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THE GERMINATION OF MOULD SPORES AT CONTROLLED HUMIDITIES

BY D. SNOW

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(With 6 Text-figures)

Observations have been made on the influence of humidity on spore germination, the rate of elongation of the germ tubes and the production of asexual and sexual fructifications of various mould fungi. The humidity range at which the different species developed is given. Some of the Mucorales and certain pathogenic moulds required high humidities (100–93%) for their successful growth. *Penicillium* spp. had a wider humidity range (100–80%). *Aspergillus* spp. were able to grow under conditions of even more restricted moisture supply. Spores of members of the *A. glaucus* group, particularly, germinated and developed at humidities as low as 70–64%. The reasons for variations in the humidity range of the different species are discussed. The influence of the supply of nutrients on spore germination and subsequent mould growth is considered.

INTRODUCTION

The factors influencing the growth of moulds on various stored products have been described by several authors. These include observations on book-binding materials by Groom & Panisset (1933), on textiles by Galloway (1935), on leather by Colin-Russ (1940), on dried meat by Macara (1943), and on feeding stuffs by Snow, Crichton & Wright (1944). It has been shown that the development of moulds on various materials is conditioned by the relative humidity of the storage atmosphere rather than by the moisture content of the materials themselves. Some of the workers mentioned above isolated different species of moulds growing on the products under observation and related the fungi obtained to the humidity of storage. It has thus been possible to obtain some knowledge of those species which can survive under conditions of restricted water supply and of those whose moisture requirement is much higher. It has usually been found impossible to observe the behaviour of individual spores present on the materials being tested. Therefore, records have generally been made of the time of appearance of mould mycelium on the samples stored at given humidities. Some observations on the influence of humidity on the germination of the spores of different species have, however, been made on glass slides by Lesage (1895), Tomkins (1929), Groom & Panisset (1933) and by Clayton (1942), and on viscose sheeting by Galloway (1935) and Chowdhury (1937). But these observations have all been made using pathogenic fungi whose moisture requirements are usually high, or the experiments have been of such limited duration (often for periods of only 1–2 weeks) that the lowest humidities at

which different moulds can grow has not been adequately determined. It should be noted, however, that the germination of conidia of some of the Erysiphaceae, powdery mildew fungi, can take place independently of the moisture content of the surrounding atmosphere (Brodie, 1945).

The aim of the experiments that are described in this paper was to determine the minimum humidities at which spores of different species would germinate. It was known that many of the moulds used were able to grow under conditions of restricted moisture supply since they had been isolated from feeding stuffs kept at controlled humidities, often as low as 70–65% (Snow, 1945). Such studies were considered to have a practical value in so far as they provided data regarding the fungi that are able to grow and flourish under different conditions of atmospheric humidity. The application of these observations to problems connected with mould deterioration of foods, textiles, leather goods, electrical and other apparatus will at once be apparent. In dealing with problems of this kind it is essential, first, to identify the species that are causing damage, and secondly, either to reduce the humidity of the place of storage to a safe level at which spore germination cannot take place or to use a suitable and effective antiseptic that will prevent mould development. Knowledge gained from such observations on the germination of the spores of pathogenic fungi at controlled humidities might also be applied to studies concerned with the environmental factors necessary for the infection of plants by certain pathogenic organisms.

EXPERIMENTAL METHODS

The majority of the fungi used in the experiments were isolated from feeding stuffs maintained at given humidities (Snow, 1945), but a few of the species used had been isolated from various natural sources on which they were living either as plant parasites or as saprophytes. The moulds belonged to the following species:

Aspergillus repens (Cda) DeBary.
A. ruber (Brem.).
A. chevalieri (Mang.) Thom & Church.
A. chevalieri var. *intermedius* Thom & Raper.
A. amstelodami (Mang.) Thom & Raper.
A. echinulatus (Delacr.) Thom & Church.
A. restrictus G. Smith.
A. candidus Link.
A. versicolor (Vuill.) Tiraboschi.
A. sydowi (Bain. & Sart.) Thom & Church.
A. niger van Tieghem.
Penicillium cyclopium Westling.
Penicillium rugulosum Thom.
P. wortmanni Klöcker.
P. phaeo-janthinellum Biourge.
P. fellutanum Biourge.
P. sartoryi Thom.
Cladosporium herbarum Link.
Tricothecium roseum Link.
Botrytis cinerea Fr.
Mucor spinosus van Tieghem.
Mucor sp.
Rhizopus nigricans Ehrenb.

Pure cultures of the moulds were obtained on agar slants. By using cultures of about 1–2 months old for the mould inoculum it was possible to obtain dry spores

of each species. The spores were sown with a sterile platinum wire on to a previously prepared base of dried gelatine. Tomkins (1929) and Groom & Panisset (1933) used plain glass slides or slides with a drop of dried nutrient agar for their studies on spore germination; Galloway (1935) and Chowdhury (1937) used thin viscose sheeting or non-moisture-proof cellophane; Lesage (1895) used plain glass slides and 'goutte de gélatine'. For the present studies it was necessary to have a base to which spores would remain attached and which would allow of their microscopic examination at regular intervals over a long period. Plain glass slides were found to be too smooth for the successful attachment of the spores. Furthermore, it was thought desirable to employ a base to which various nutrients could be added to encourage the germination and subsequent growth of the spores. These conditions were satisfied by using sheet gelatine cut into half-inch squares and dipped for 3–4 sec. in warm distilled water or some nutrient solution such as beer-wort (which contains maltose sugar) at 50° C. The heat treatment partially dissolved some of the surface of the squares of gelatine which could then be affixed to glass slides and smoothed down with a spatula. The gelatine squares were dried for 48 hr. at laboratory temperature so that the surface of the gelatine became quite smooth, hard and dry. Spores of the moulds could then be readily streaked across the dry gelatine squares with a platinum wire and remained fixed so that detailed observation could be made with both the low ($\times$ 100) and higher ($\times$ 450) powers of the microscope.

When the spores of the species under examination had been sown, the glass slides with the dried gelatine squares were placed in small cylindrical museum jars at a range of controlled atmospheric humidity (R.H.). This was regulated in two ways: first, by saturated salt solutions using the data of Spencer (1926) and, secondly, by sulphuric acid solutions of known specific gravity (Wilson, 1921). Both these methods of humidity control proved satisfactory for the purpose of the experiments and gave comparable results. The museum jars in which the humidities were controlled by saturated salt solutions were maintained at 20° C. and those in which sulphuric acid solutions were used at 25° C. Sulphuric acid was preferred as a means of controlling the humidity since a more regular and comprehensive range of humidity intervals could be obtained more easily than with the different saturated salt solutions. Provided that the acid solutions are pure and free from introduced organic matter no danger exists from acid fumes since sulphuric acid is non-volatile. The glassware used in the experiments was sterilized in a hot air oven at 130° C. for 5 hr.

Water was absorbed by the gelatine squares presumably until an equilibrium had been reached between the water content of the thin squares of gelatine and the controlled atmospheric humidity in the museum jars. To outward appearances the gelatine squares maintained at high humidities (100–90%) absorbed relatively large amounts of water so that they became moist and sticky to the touch, while at lower humidities (below 90%) the squares remained smooth, hard and dry. It is not considered likely that the water content of the gelatine would substantially influence

the rate of germination because, as stated previously, this rate has been shown to depend on the atmospheric humidity rather than on the moisture content of the substrate. Moreover, since the gelatine squares were thoroughly dried in the laboratory before being introduced to the controlled humidities, the dry mould spores could not have obtained access to water from the gelatine strips in excess of that determined by the equilibrium maintained by the humidity of the atmosphere in which they were exposed.

The glass slides with the gelatine squares were removed from the museum jars at regular intervals of from 1 day upwards for microscopic observation of the mould spores. Adopting the criteria suggested by Tomkins (1932) the number of days before spore germination took place (latent period of spore germination) was calculated and the rate of elongation of the germ tubes was determined from an average of the measured length of twenty germ tubes on the spore streak. The spores were selected at random except that those with branched germ tubes were avoided as also were those that were not lying singly on the gelatine but were germinating from a clump of spores. It was found that, provided only unbranched germ tubes were taken into account, accurate measurements of up to 120 μ could be made of all the species tested. Beyond this length the frequency of branching of the germ tubes prevented accurate measurements being recorded. For some species the percentage germination of the spores was noted. In addition to observations on the growth of the vegetative germ tubes, the latent periods before the production of asexual fruiting bodies (conidiophores or sporangiophores) and before the production of sexual fruiting bodies (yellow cleistothecia of the *Aspergillus glaucus* group) were measured.

RESULTS

The influence of humidity on the germination of conidia of Aspergillus repens

Conidia of *A. repens*, which is known to be one of the most common moulds on stored feeding stuffs (Snow, 1945), were sown on dried gelatine squares without additional nutrients. Duplicate slides were maintained at humidities of 100, 98, 95, 90, 88, 84, 81, 78, 76, 72, and 66% regulated by saturated salt solutions at 20° C. Within the period of the experiment (210 days) spore germination had taken place at all humidities between 100 and 72% but not at 66% R.H. The rate of germination (as shown by the latent period of spore germination) was found to be conditioned by the humidity of the atmosphere (Fig. 1). At high humidities (over 88%) germination took place relatively quickly, i.e. in 2 or 3 days, but at humidities below 88% the latent period of spore germination was much longer. At 76% R.H. it amounted to over 30 days at 20° C.

The rate of elongation of the germ tubes of *A. repens* was also conditioned by the relative humidity of the atmosphere. At 100–90% R.H. rapid elongation took place which is shown by an almost vertical straight line (Fig. 2). As the water content of the atmosphere is reduced from 90 to 80% R.H. the slope of the graph tends more

away from the vertical indicating a slower but still a straight linear growth rate. Below 80% R.H., however, the rate of elongation of the germ tubes is markedly checked so that the growth rates of the germ tubes can no longer be represented by a straight line but assume a curved form.

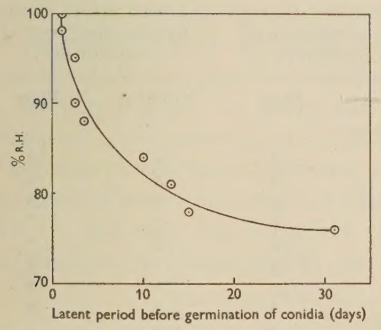

Fig. 1. The influence of humidity on the germination of conidia of *Aspergillus repens* on plain gelatine at 20° C.

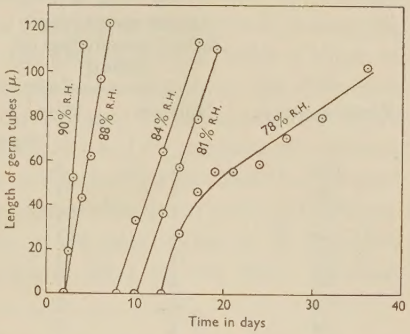

Fig. 2. The influence of humidity on the growth of germ tubes of *A. repens* on plain gelatine at 20° C.

The influence of nutrients on spore germination and growth of Aspergillus repens *and* A. chevalieri

Two series of experiments were made. In the first the germination and subsequent growth of the conidia of *A. repens* and *A. chevalieri* were compared on plain dried gelatine squares and on gelatine squares which had been dipped in warm beer-wort before drying (nutrient gelatine). These were kept at a series of fixed humidities between 100 and 76% maintained by sulphuric acid solutions at 25° C. In the second the growth and development of conidia and ascospores of *A. chevalieri* were compared on plain and nutrient gelatine at humidities between 100 and 84% maintained by saturated salt solutions at 20° C. Measurements were made to determine the latent period of spore germination, the growth rate of the germ tubes, and the latent period before the development of asexual fruiting bodies (conidiophores) and before the development of sexual fruiting bodies (yellow cleistothecia). The results for the 25° C. series, given in Table 1, show that the humidity determined the rate of germination and subsequent growth of both the species tested. This confirmed the observations made with *A. repens* in the experiment described previously. Fig. 3 shows that, for *A. chevalieri*, the humidity determined not only the rate of spore germination at 25° C. but also the latent period before the production of both asexual and sexual fruiting bodies. The figures obtained for the latent period of spore germination and subsequent mould development in the second series, where the humidity was controlled by saturated salt solutions at 20° C., were similar to those recorded in the first series. Where differences in the figures existed they indicated that the rate of mould growth was slower at 20

than at 25° C. and, in addition, that mould development from ascospores proceeded at a slightly slower rate than from conidia.

TABLE 1. *The influence of nutrient and plain gelatine on spore germination and the growth of moulds at given humidities at 25° C.*

	Latent period (days) before germination of conidia		Latent period (days) before appearance of conidiophores		Latent period (days) before appearance of cleistothecia	
Percentage R.H.	Nutrient	Plain	Nutrient	Plain	Nutrient	Plain
			Aspergillus repens			
100	1	1	2	3	4	12
95	1	1	2	4	4	19
93	1	1	2	5	6	56
90	1	1	3	7	7	*
88	2	2	4	10	10	*
86	2	7	5	25	19	*
84	2	7	7	57	*	*
82	3	7	12	*	*	*
80	4	7	13	*	*	*
78	6	8	30	*	*	*
76	14	16	*	*	*	*
			A. chevalieri			
100	1	1	2	4	3	12
95	1	1	2	4	4	19
93	1	1	2	4	6	36
90	2	2	3	5	8	56
88	2	3	3	10	9	*
86	2	5	5	14	14	*
84	3	7	7	19	19	*
82	5	7	12	25	*	*
80	7	7	15	46	*	*
78	10	10	20	*	*	*
76	19	23	*	*	*	*

* Fructifications had not appeared at 56 days.

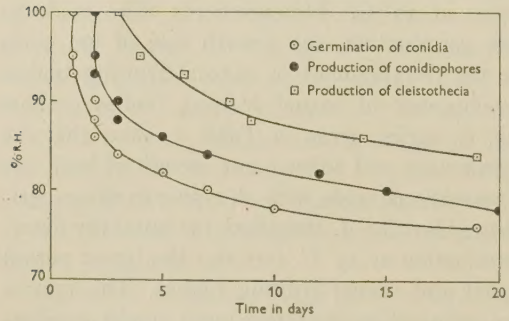

Fig. 3. The influence of humidity on the germination and growth of *A. chevalieri* on nutrient gelatine at 25° C.

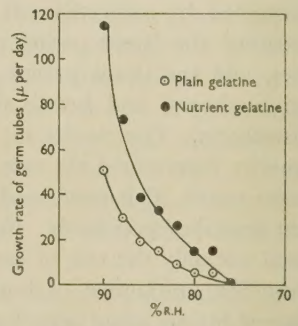

Fig. 4. The influence of nutrients on the growth rate of germ tubes of *A. chevalieri* at controlled humidities.

The influence of nutrients on spore germination can be clearly seen in Table 1. Tomkins (1929) found that the presence of nutrients decreased the latent period of spore germination for the species tested. The findings of the present series of experiments show that there is some indication that this may also hold true for moulds of the *A. glaucus* group since, below 90% R.H., the times given for the latent period of spore germination on nutrient gelatine were generally shorter than those on plain gelatine (Table 1). But as the figures in the first column of Table 1 show, at humidities above 90% and for some measurements below 80% R.H., this difference was not very marked. It seems likely that the primary factor controlling spore germination is the water content of the atmosphere and that the presence of nutrients is of secondary importance. However, once the conidia had germinated, the presence of nutrients exerted a more marked effect on the rate of elongation of the germ tubes. It will be seen from Fig. 4 that the elongation of the germ tubes of *A. chevalieri* took place much more rapidly on the nutrient than on the plain gelatine.

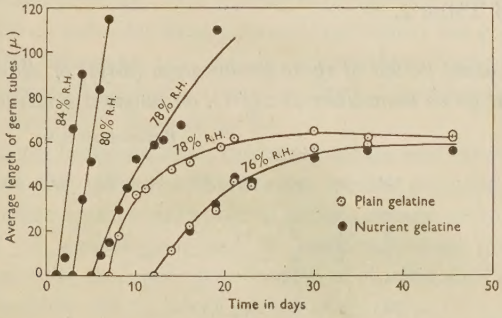

Fig. 5. The influence of humidity on the growth of germ tubes of *A. repens* on plain and nutrient gelatine at 25° C.

Further, the development of both asexual and sexual fruiting bodies was not only hastened by between 2 and 9 times on the nutrient gelatine but also took place at lower humidities than on the plain gelatine (Table 1). Dense masses of well-developed conidial heads were produced on the nutrient gelatine but sporulation on the plain gelatine was very meagre, often only a single chain of conidia being produced from each conidiophore. Nutrients also influenced the development of the sexual fruiting bodies. Where beer-wort nutrients were provided yellow cleistothecia appeared in profusion within a relatively short time, but on the plain gelatine their development was almost entirely checked even at the highest humidities. This would therefore support the statement of Thom & Raper (1945) that the formation of cleistothecia is favoured by an abundance of assimilable carbohydrate.

As with the previous experiment the rate of elongation of the germ tubes of *A. repens* can be expressed by a straight line at humidities of 80% and above (Fig. 5). Below 80% R.H. the line representing the growth rate tends to become more

curved. At 78% R.H. this is much more marked for the spores germinating on the plain than for those on the nutrient gelatine. This indicates that, at this humidity, nutrients play an important part in maintaining the growth of this species. At 76% R.H., however, both the plain and nutrient gelatine curves assume almost identical form and can only be represented by a single line of the graph. This suggests that even the presence of nutrients is insufficient to prevent the limited amount of moisture in the atmosphere at this reduced humidity checking the growth of the germ tubes.

The influence of humidity on the germination of spores of different moulds on nutrient gelatine

The spores of a range of moulds, listed in Table 2, were sown on dried nutrient gelatine squares and exposed to controlled humidities between 100 and 71% at 25° C. As before the latent period of spore germination was measured and the results are given in Table 2.

TABLE 2. *Latent period of spore germination (days) of different moulds at given humidities at* 25° C. *on nutrient gelatine*

	Percentage R.H.												
	100	95	93	90	88	86	84	82	80	78	76	75	73
Mucor spinosus	1	1	2	—	—	—	—	—	—	—	—	—	—
Mucor sp.	1	1	2	—	—	—	—	—	—	—	—	—	—
Rhizopus nigricans	1	1	2	—	—	—	—	—	—	—	—	—	—
Botrytis cinerea	1	2	2	—	—	—	—	—	—	—	—	—	—
Tricothecium roseum	1	1	2	4	—	—	—	—	—	—	—	—	—
Cladosporium herbarum	1	1	2	2	7	—	—	—	—	—	—	—	—
Penicillium rugulosum	—	—	—	3	4	9	—	—	—	—	—	—	—
P. cyclopium	1	1	2	2	3	4	9	—	—	—	—	—	—
Aspergillus niger	1	1	2	2	3	4	11	—	—	—	—	—	—
Penicillium phaeo-janthinellum	—	—	—	3	3	4	7	15	—	—	—	—	—
P. wortmanni	—	—	—	2	2	3	4	20	—	—	—	—	—
Aspergillus nidulans	—	—	—	1	2	2	4	18	—	—	—	—	—
Penicillium sartoryi	—	—	—	2	2	4	4	12	18	—	—	—	—
P. fellutanum	—	—	—	—	2	4	4	9	12	—	—	—	—
Aspergillus versicolor	—	—	—	1	2	2	4	7	9	24	—	—	—
A. sydowi	—	—	—	1	2	2	4	7	12	34	—	—	—
A. chevalieri var. *intermedius*	—	—	—	—	—	—	—	4	7	12	24	53	—
A. restrictus	—	—	—	—	—	—	—	3	4	7	12	15	—
A. candidus	—	—	—	—	—	—	—	4	7	9	12	15	—
A. amstelodami	—	—	—	—	—	—	—	4	4	9	12	18	—

In contrast to the previous experiments, when two species whose spores were able to germinate at a wide humidity range were used, many of the moulds tested in this experiment had a much more restricted range of humidity. Thus spores of the Mucorales were able to germinate only at humidities between 100 and 93%. The same was true for spores of the grey mould, *Botrytis cinerea*. The imperfect fungi,

Tricothecium roseum and *Cladosporium herbarum*, had a slightly wider range of humidity over which spore germination was possible. These species had critical humidities of 90 and 88%, respectively.

Penicillium spp. had an even wider humidity range and the lowest level at which spore germination was observed varied from 86–80% R.H. for different species. Conidia of the biverticillate *P. rugulosum* did not germinate at humidities below 86% even after a latent period of 81 days. On the other hand, the critical humidity for the monoverticillate *P. sartoryi* and *P. fellutanum* was 80%.

The Aspergilli also showed variation in their ability to grow at different levels of humidity. *Aspergillus niger* had the most restricted range of humidity (100–84%) over which spore germination was possible; conidia of *A. nidulans* were able to germinate at 100–82% R.H., while spores of the two members of the *A. versicolor* group (*A. versicolor* and *A. sydowi*) germinated at humidities of 78% and above. *A. candidus* appeared to have a very wide range of humidity (100–75%) over which spore germination was possible. This finding is in agreement with that of Galloway (1935) who included *A. candidus* among those fungi which can grow at relatively low humidities and who also gave 75% as the critical humidity for this species. In addition it may be noted that Snow (1945) isolated *A. candidus* from feeding stuffs stored at various humidities down to 70%.

It was quite evident, however, from the experiments made that the moulds whose humidity range was the most extensive were species included in the *A. glaucus* group. Spores of these species were able to germinate and to flourish at humidities well below 80%. *A. amstelodami* and *A. restrictus* could grow successfully at humidities as low as 75% and spore germination of *A. chevalieri* var. *intermedius* was possible at this humidity after a latent period of 53 days.

The germination of conidia of species of the Aspergillus glaucus *group at low humidities*

It seemed desirable at this stage to establish the minimum humidities at which spore germination of species of the *A. glaucus* group could take place since these would probably indicate the lowest levels at which mould fungi could grow. Accordingly, four species were chosen whose moisture requirements were known to be very low. The moulds had been isolated from feeding stuffs stored at fixed humidities for periods up to 4 years (Snow, 1945). The moulds used were as follows:

A. chevalieri isolated from linseed cake stored at 85% R.H.
A. repens from oats at 67% R.H.
A. echinulatus from locust beans at 65% R.H.
A. ruber from locust beans at 70% R.H.

Dry conidia of each of the moulds growing on agar slopes were sown on dried nutrient gelatine squares fixed to glass slides. The slides were then exposed to a range of low humidities at close intervals between 75 and 60% maintained by

sulphuric acid solutions at 25° C. Observations were made regularly to determine the latent period of spore germination and the rate of elongation of the germ tubes at the various humidities. Spore germination of all four species took place readily at 75 and 73% R.H. after latent periods of 1–4 weeks. The critical humidity below which the conidia did not germinate within the initial period of the experiment (200 days) for *A. chevalieri* was 73%, but conidia of *A. repens* and of *A. echinulatus* were able to germinate at 71% R.H. after latent periods of 92 and 43 days, respectively, and a small percentage of the conidia of *A. ruber* germinated at a humidity as low as 70% after a latent period of 120 days. It was noted that ascospores of *A. echinulatus* which were sown by chance along with the conidia on the gelatine squares germinated equally well at the lower humidity levels. After spore germination the germ tubes continued to lengthen although at a much slower rate than those which had been measured previously at humidities above 75%. At 73% R.H. the rates of elongation of the germ tubes of the four moulds were calculated to be between 3 and 5 μ per day; at 71% R.H. the germ tubes of *A. ruber* and *A. echinulatus* took approximately 80 days after spore germination to reach an average length of 85 μ—an average rate of elongation of about 1 μ per day. The rates of growth of the germ tubes of *A. repens* at 71% and of *A. ruber* at 70% were even slower.

Many of the germ tubes produced at such low humidities were badly misshapen, often with swollen and thickened tips. This suggested that the spores had been induced to produce germ tubes at lower humidities than were generally favourable to their successful survival and continued growth and that, in order to adapt themselves to conditions of low moisture supply, they developed swollen and thick-walled chlamydospore structures which were more resistant to the dry conditions than were the normal thin-walled germ tubes. From the observations made, however, it is clear that, of the four species of the *A. glaucus* group tested, *A. ruber* and *A. echinulatus* are the most resistant to conditions of low humidity, followed by *A. repens* and *A. chevalieri*.

In view of the observations of Wright (1940) and Snow *et al.* (1944) that feeding stuffs stored at humidities of 65–70% often showed no signs of moulding until a latent period of 1–2 years had elapsed it was decided to maintain some of the spores of the four species on nutrient gelatine squares at fixed humidities between 60 and 70% for longer than the initial period of 200 days. These were examined periodically for spore germination. Of the four species *A. echinulatus* showed the greatest ability to germinate at such low humidities provided that an extended latent period was given. After almost 2 years a small proportion of the conidia of this species had germinated at 66, 65 and 64% R.H. and one or two conidia had even produced germ tubes at 62% R.H. Many of the germ tubes produced were misshapen and probably not viable, but a few were able to grow to lengths of more than 100 μ. It is suggested, therefore, that of all the moulds tested this species shows the greatest adaptation to conditions of restricted moisture supply.

General conclusions

The experiments described have shown that of the moulds tested each species has a given range of humidity at which its spores can germinate. These are shown in Fig. 6.

The spores of some of the Mucorales can only germinate and develop extensive mycelia where the atmospheric conditions approach saturation. Pathogenic moulds, such as *Botrytis cinerea*, the grey mould, have similarly high moisture requirements. Moulds such as *Tricothecium roseum* and *Cladosporium herbarum* are less exacting in their moisture requirements having critical humidities of 90 and 88% respectively. *Penicillium* spp. are quite suited to withstand certain degrees of sub-saturated atmosphere and spore germination can continue at humidities above the critical limit of 86–80% according to the species. The same holds true for a number of the Aspergilli. *Aspergillus niger*, *A. nidulans*, *A. versicolor* and *A. sydowi* were found to have a similar range to many of the Penicillia.

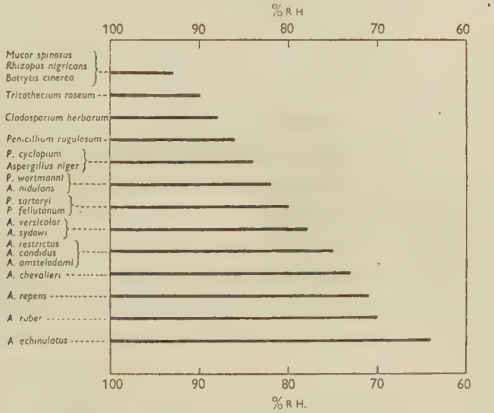

Fig. 6. The range of humidity for spore germination on nutrient gelatine at 25° C.

It is clear that, of all the moulds tested, the *A. glaucus* group have the lowest moisture requirements for spore germination and subsequent growth of the germ tubes, as well as for the formation of mould mycelium and the production of both asexual and sexual fruiting bodies. Of the small ascospored species of this group, which are so commonly found causing deterioration of a wide range of stored products, *A. ruber* was the most resistant mould to conditions of low humidity. Conidia of this species were able to germinate and produce active germ tubes at humidities as low as 70% within a latent period of 4 months. *A. repens*, *A. chevalieri* and *A. amstelodami*, in this order, were able to acclimatize themselves readily to humidities of 75–73% which enabled a small percentage of their spores to germinate and maintain growth. The less common large ascospored species, *A. echinulatus*, was

able to flourish under conditions of low humidity and both conidia and ascospores of this species could easily germinate at 71 % R.H. Evidence has also been presented which shows that, if a sufficiently extended latent period (1–2 years) is given, a small percentage of the conidia of *A. echinulatus* will germinate at humidities as low as 66–62 %. The ability of species of the *A. glaucus* group to exist at low humidities will inevitably lead to their very frequent isolation from a wide variety of products stored at sub-saturated atmospheres and to the exclusion of other moulds which are not so well adapted.

The reasons why some moulds are better able to withstand conditions of low humidity than others are not fully understood. It is evident that members of the *A. glaucus* group are the most highly adaptable in this respect. Certainly the spore walls of *A. ruber* and *A. echinulatus* are very thick and spinulose compared with the thin smooth walls of the spores of most members of the Mucorales, *Botrytis cinerea* and many *Penicillium* spp. The same holds true, to a lesser extent, for *Aspergillus repens* and *A. amstelodami*. The protection afforded by a thick spore wall would conceivably enable the mould to withstand periods of long desiccation. But the ability of spores of species included in the *A. glaucus* group to absorb sufficient water for germination through the spore wall when exposed to sub-saturated atmospheres of the order of 75–65 % R.H. suggests that the osmotic pressure of their cell sap must be very much higher than that of the majority of other moulds.

It seems likely that such absorption can only take place at an extremely slow rate since latent periods of up to 2 years have been measured before germination at these low humidities occurred. It is possible that the spores require an extended latent period before they become acclimatized and able to germinate under conditions of such restricted moisture supply. But, inevitably, the time during which the protoplasm in the dormant spores can remain viable must be limited. An indication of this time was given by transferring the slides of spores of the four species that had been maintained at 60 % R.H. for almost a year without showing any signs of growth to a higher humidity (80 %) at which germination could more readily take place. Germination of all four species occurred at the higher humidity within 2 weeks. The viability of spores of members of the *A. glaucus* group can therefore be maintained for at least a year at 60 % R.H. and at 25° C. Beyond this time, however, the percentage viability of spores will inevitably be reduced and a high proportion will never achieve successful germination if maintained under these environmental conditions.

The observations have indicated that young germ tubes require a higher humidity for their subsequent active healthy growth than is essential for the early stages of germination from the dormant spore. Where this is not provided misshapen chlamydospore structures are produced by the germ tubes. Thus, although spore germination of species of the *A. glaucus* group may occur between 70–60 % R.H. active mycelial growth is not likely to be extensive within this range of humidity. It may, therefore, be concluded that while mould growth must always be regarded

as a potential source of damage on products stored for long periods at 70–60 % R.H., its extensive development is unlikely unless the humidity exceeds 70%.

In addition to the part played by humidity, some indication has been given that the presence of nutrients may hasten the rate of spore germination. The experiments clearly indicated that, after spore germination had occurred, the presence of beer-wort nutrients encouraged the elongation of the germ tubes and the production of both asexual and sexual fruiting bodies. Indications have also been given that the atmospheric temperature influences the rate of spore germination and subsequent mould development.

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A NECROTIC DISEASE OF FORCED TULIPS CAUSED BY TOBACCO NECROSIS VIRUSES

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(With Plate 1)

Tobacco necrosis viruses were found to be the cause of severe necrotic disease which occurred in forced tulips in three different nurseries. From 20 to 50% of some varieties, e.g. Alberio, Korneforus, Crater, Rose Copland and Krelage's Triumph were affected, whereas others growing under the same conditions remained apparently healthy. The characteristic symptoms of the disease are necrotic spots and streaks, which cause much stunting and twisting and soon kill small plants. The disease was reproduced by inoculating healthy tulips with tobacco necrosis viruses.

A necrotic condition of tulip plants raised under glass was first noticed during 1940 in three different English counties and was described and figured by Moore (1946). No pathogen was identified and the injury was attributed to the effects of frost immediately before housing.

Specimens of forced tulips with apparently identical symptoms were received at Rothamsted in February and March 1948 from Mr W. Buddin and Dr W. A. R. Dillon Weston. The disease was found in three different nurseries, where it caused considerable losses, from 20 to 50% of the plants being killed. Alberio, Korneforus, Crater, Rose Copland and Krelage's Triumph were all affected, while other varieties growing in the same houses remained apparently healthy. All the plants in the houses, whether diseased or healthy, were raised from bulbs newly imported from Holland. The necrotic plants were all found to be infected with tobacco necrosis viruses which, although known to have a wide host range, have not previously been recorded as causing an economic disease.

The name tobacco necrosis was first used by Smith & Bald (1935) for a virus disease of tobacco seedlings in which the lowest leaves became necrotic. Later Smith (1937) showed that the roots of tobacco plants and many other species were often infected though the leaves showed no symptoms and were apparently virus-free. Many different plants were found to be susceptible, but in none was infection systemic. Except *Primula obconica*, which is a symptomless carrier (Bawden & Kassanis, 1947), all other known susceptible plants are hypersensitive, and when inoculated react by producing necrotic lesions only.

Tobacco necrosis can be caused by several viruses which are serologically unrelated and also differ in particle size and certain other properties (Bawden, 1941; Bawden & Pirie, 1942, 1945). There is no record of these viruses occurring in the

roots of plants grown in the field; in the roots of plants grown in glasshouses they occur most frequently in the winter months.

Symptoms

There is no mottling and symptoms consist exclusively of necrotic spots and streaks, which are most common on the leaves but also occur on stems and flowers. The naturally-infected tulips showed considerable differences in severity of symptoms, particularly in the amount of the plant to become necrotic. Small plants were often acutely necrotic and soon collapsed completely, whereas in large plants the necrosis was less general and spread more slowly. In most plants, however, infection sooner or later led to the death of the whole plant. In some plants the necrotic areas were numerous and relatively small, while in others they were few and large. The latter were especially common in plants that first produced symptoms when their flowers were developed, and the necroses were then often confined to one-half of the leaf, on which necrotic streaks extended the whole length (Pl. 1, fig. 1). Younger plants had necrotic areas covering their whole surface and the leaves were stunted, twisted and shrivelled (Pl. 1, fig. 2); such plants rarely flowered, though occasionally they carried a much twisted flower stalk with a small ill-formed bloom (Pl. 1, fig. 4). In some plants, which seem to have been invaded when more mature, symptoms were restricted to a withering of the flower and an occasional necrotic streak on the leaves or flower stalk. As with the leaves, infection of the flowers was occasionally on one side only, so that half of the petals were deformed and necrotic whereas the others appeared normal. A few infected flowers were not malformed and showed symptoms consisting of a fine necrosis running along the veins (Pl. 1, fig. 5).

Many of the infected flowers had unusual numbers of floral parts. The most common was 8 petals, 8 stamens and 4 lobes to the stigma instead of the usual 6—6—3, but other combinations such as 6—7—3, 8—6—3, 10—10—3, 7—9—4, 6—7—4, 7—7—4 were also seen. Whether these abnormalities were caused by the infection, however, is not certain, for some other plants of the varieties Alberio and Korneforus were found (Pl. 1, fig. 3) which also had withered flowers and similar abnormal numbers of floral parts although no viruses could be obtained from them; also, similar abnormalities have been attributed to storage at low temperatures (Purvis, 1938).

Infectivity and serological tests

Inocula from diseased tulips from all three nurseries produced the characteristic local lesions of tobacco necrosis when rubbed with the aid of abrasive on to leaves of *Phaseolus vulgaris*, *Nicotiana tobacum* and *N. glutinosa*. Using *P. vulgaris* (variety Prince) as a test plant, virus was demonstrated in the roots, bulbs, leaves and flowers of diseased tulips. The viruses were not uniformly distributed throughout all the tissues of infected plants. Leaves free from symptoms contained no demonstrable virus, which occurred most abundantly in necrotic tissue, though it was also present in apparently normal tissue of leaves that bore a few necrotic streaks.

The virus was always present in the roots and in the main and axillary buds of the bulbs. In young roots brown lesions were often seen and inoculum from these produced many more lesions on beans than inoculum from the rest of the roots. The buds of the bulbs, however, looked normal though the virus was present, sometimes at concentrations even higher than in the roots.

The leaf symptoms in tulips were reproduced by inoculating healthy tulips, with the aid of an abrasive, with sap from infected tulip or tobacco. In one test, ten plants in a box containing twenty healthy-looking tulips (variety Rose Copland) were rubbed with inoculum taken from infected tobacco plants. A week later a few necrotic elliptic spots appeared in the inoculated leaves. They spread along the veins to form streaks, which ultimately coalesced to produce symptoms resembling those of the naturally-infected plants. Later, necrotic streaks also appeared in the uninoculated younger leaves. The plants in the other half of the box remained healthy until maturity. Similar results were obtained with varieties Alberio and Korneforus inoculated with sap from infected tulips.

To find whether the virus from tulips was a new one or related to any previously described, serological tests were made with antisera prepared against the tobacco necrosis viruses studied by Bawden & Pirie (1942, 1945). The serological tests were made with sap from tobacco plants in which virus from four infected tulips was separately propagated. Partially purified preparations were used to avoid unspecific precipitation. The sap was one-third saturated with ammonium sulphate, centrifuged and the precipitate dissolved in a volume of water equal to one-fifth of the original volume of sap. The solution was adjusted to pH 4·5, centrifuged and the precipitate discarded. The supernatant fluid was brought to pH 6·5 and used in the precipitin tests.

TABLE 1. *Serological reaction of four different virus isolates from diseased tulips*

	Isolates from			
Antisera against	Nursery A tulip 1	Nursery A tulip 2	Nursery A tulip 3	Nursery B tulip 4
A mixture of tobacco necrosis viruses	+	+	+	+
Potato culture of tobacco necrosis virus	−	+	−	−
Culture II of tobacco necrosis virus	−	−	−	−
Rothamsted culture of tobacco necrosis virus	−	−	+	+
Saline control	−	−	−	−

Table 1 shows that all the diseased tulips contained viruses serologically related to those previously called tobacco necrosis. The results also show that the tulips were not all infected with the same virus, but that a number of them can produce the same malady. Actually some tulips growing in the same nursery were infected with at least two different viruses whereas some tulips from different nurseries contained the same virus.

In addition to the serological tests with partially purified sap from infected tobaccos, tests were also made with sap from naturally-infected tulips. No reaction

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Photographs by V. Stansfield, F.R.P.S.

was obtained with most, but with some plants that were severely affected there was enough virus in the sap to give precipitin reaction at a dilution of 1:2.

In glasshouses where the outbreaks occurred only certain varieties were affected. This may mean that different varieties differ in their susceptibility to tobacco necrosis viruses, but positive evidence for this must await the results of experimental tests. It is also unknown whether the viruses infected the diseased plants during the current or the previous season. There seems little doubt that tobacco necrosis viruses are soil-borne, but how they survive and enter healthy plants is unknown. In most naturally-infected plants the viruses are apparently restricted to the roots, and tulips are the first hosts to be recorded in which, presumably entering through the roots, they have permeated the aerial parts.

I wish to thank Mr W. Buddin and Dr W. A. R. Dillon Weston for providing me with material and much useful information.

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EXPLANATION OF PLATE 1

Fig. 1. A leaf of naturally infected tulip (variety Alberio), showing a single broad necrotic streak extending from base to tip.

Fig. 2. Tulip (variety Alberio) naturally infected with a tobacco necrosis virus and showing numerous necrotic spots and streaks.

Fig. 3. Tulip (variety Alberio) with chlorotic leaves and withered flower. No virus was recovered though similar flower symptoms also occur in tulips infected with tobacco necrosis viruses.

Fig. 4. The flower stalk of a naturally infected tulip (variety Korneforus) heavily streaked and twisted, with the flower severely stunted, malformed and necrotic.

Fig. 5. Flower of naturally infected tulip (variety Korneforus), showing very fine necrosis-like etching along the veins on one side of the petals.

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RESOLUTION OF STRAWBERRY VIRUS COMPLEXES

III. THE ISOLATION AND SOME PROPERTIES OF VIRUS 3

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(With Plate 2)

Aphids (*Capitophorus fragariae* Theob.) allowed to feed for several days on a strawberry plant with severe crinkle transmitted two viruses. The isolation and properties of one (virus 1) have already been described. The other (virus 3) was separated by transferring the aphids to fresh indicators after 24 hr.

Virus 3 was transmitted by aphids which had been allowed to feed on an infected plant for 6 days or more and persisted in the vector for several days. There was some evidence that the virus has a latent period in the vector. The symptoms produced by virus 3 on *Fragaria vesca* and Royal Sovereign strawberry are described.

On Royal Sovereign, viruses 1 and 3 together produced symptoms of severe crinkle and viruses 2 and 3 together produced yellow-edge. A form of severe crinkle is thus shown to be caused by a virus complex which can be resolved by means of the vector, and severe crinkle is shown to be etiologically distinct from mild crinkle.

Introduction

The separation (by means of *Capitophorus fragariae*) of one virus (virus 1) from the virus complexes occurring in strawberry plants showing symptoms of mild crinkle, severe crinkle and yellow-edge has been reported (Prentice & Harris, 1946). A second virus (virus 2) was isolated from plants with yellow-edge (Prentice, 1948) and the present paper describes the isolation of a third (virus 3) from plants with severe crinkle.

A preliminary account of some of the work has already been published (Prentice, 1946). The methods employed were similar to those previously described (Prentice, 1948) and are summarized below.

Aphids (*C. fragariae* Theob.) were allowed to feed for a predetermined time (the *infection feeding period*) on an infected plant (the *infector*) and then transferred to a series of indicator plants. After the appropriate period (the *first transfer feeding period*) the aphids were retransferred to a fresh set of indicators for the second transfer feeding period and, in some experiments, subsequently retransferred to further series of indicator plants. The number of plants infected in each experiment is shown as a ratio, the numerator being the number of plants that developed symptoms and the denominator the number receiving a particular treatment.

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Experimental

(i) *Isolation of virus* 3 *and symptoms on* Fragaria vesca

Virus 3 was isolated in the third and fourth transfers in Experiment 1, made in July 1943 (see Table 1).

Table 1. *Experiment* 1 (*July* 1943)

Infector: Royal Sovereign plant with severe crinkle.
Infection feeding period: 12 days.
Indicator: F. vesca. Four aphids per indicator.

Transfer	Period	Plants infected
1	24 hr.	3/4
2	24 hr.	0/4
3	24 hr.	1/4
4	24 hr.	1/4

Symptoms appeared on the indicators of the first transfer feeding period after 2–3 weeks and were of the type produced by virus 1 (Prentice & Harris, 1946). Symptoms developed on indicators of the third and fourth transfer feeds about 8 weeks after the original transfers and were of an entirely different type. Conspicuous, scattered, interveinal, chlorotic spots appeared on the leaflets which were downcurled, twisted and severely distorted (Pl. 2, figs. 1 and 2). Later the centres of some of the chlorotic spots became necrotic and red. Short lengths of the veins were also necrotic, the necrotic regions being yellow or brown. In later experiments the first symptoms of infection were noted in 4–6 weeks and it was found that the stolons on infected plants often became thickened, flattened and shortened. In some plants greenish patches, up to 1 cm. in length, developed on the stolons (which are normally red) and such patches often split or became necrotic. After about a month the symptoms gradually became less severe and the plants recovered somewhat. The virus producing the symptoms described will be referred to as virus 3.

A second experiment gave similar results (see Table 2).

Table 2. *Experiment* 2 (*July* 1943)

Infector: Royal Sovereign with severe crinkle (as Exp. 1).
Infection feeding period: 10 days.
Indicator: F. vesca. Five aphids per indicator.

Transfer	Period	Plants infected
1	48 hr.	5/8
2	48 hr.	1/8
3	48 hr.	1/8

In Exp. 2 the symptoms on the indicators of the first transfer feed resembled those caused by virus 1, while those on the indicators of the second and third transfer feeds were of the new type produced by virus 3.

(ii) *Symptoms on Royal Sovereign*

Virus 3, when transmitted to virus-free Royal Sovereign plants by grafting, produced prominent chlorotic spots on the leaves and, sometimes, necrosis along short lengths of the smaller veins. Later some of the chlorotic spots became necrotic and dark red-brown and there was some distortion and 'crinkling' of the leaves. The symptoms produced by virus 3 on Royal Sovereign thus resemble a mild form of 'severe crinkle' as met with in the field. After some months the symptoms gradually became milder, but again became more severe in the early summer, although they were never again as severe as when they first appeared.

Similar symptoms were produced on Royal Sovereign plants infected with virus 3 by means of aphids (see Tables 3 and 4), but in some plants vein clearing of short lengths of the smaller veins and purple spotting on the leaf surface was noted before the typical chlorotic spotting and necrosis developed.

TABLE 3. *Experiment* 3 (*July* 1943)

Infector: Royal Sovereign with severe crinkle (as Exp. 1).
Infection feeding period: 10 days.
Indicator: Royal Sovereign. Four aphids per indicator.

Transfer	Period	Plants infected
1	24 hr.	3/4
2	24 hr.	1/4
3	24 hr.	1/4
4	24 hr.	2/4

TABLE 4. *Experiment* 4 (*August* 1943)

Infector: Royal Sovereign with severe crinkle (as Exp. 1).
Infection feeding period: 6 weeks.
Indicator: Royal Sovereign. Four aphids per indicator.

Transfer	Period	Plants infected
1	24 hr.	5/8
2	24 hr.	1/4

The symptoms on the indicators of the second and subsequent transfer feeds in Exps. 3 and 4 were similar to those produced by virus 3 (described above) and developed in 4–6 weeks. Symptoms on the indicators of the first transfer feeds were of the same general type but were rather more severe. Such symptoms may have been due to the joint effect of virus 1 and virus 3 or to infection with some other combination of viruses.

(iii) *Transmission*

Virus 3 was retransmitted to *F. vesca* after infection feeding periods of 6 days or more (see Table 5).

TABLE 5. *Transmission of virus 3 after different infection feeding times (Data from several trials, 1945 and 1946)*

Infector: Royal Sovereign infected with virus 3.
Infection feeding period: Various.
Indicator: F. vesca. Five aphids per indicator. Single transfer.

	Infection feeding period					
Trial	1 day	4 days	6 days	8 days	12 days	16 days
A	—	0/5 (1)*	—	0/5 (1)	0/5 (1)	3/5 (1)
B	—	0/5 (8)	—	0/5 (7)	2/5 (7)	3/5 (3)
C	—	0/5 (8)	—	3/10 (8)	4/5 (4)	0/5 (1)
D	0/5 (9)*	—	2/5 (9)	—	—	—
Totals	0/5	0/15	2/5	3/20	6/15	6/15

* The period (in days) for which the aphids were allowed to feed on the indicators (i.e. the first transfer feeding period) is shown by the figure in brackets.

It will be seen from Table 5 that, with infection feeding periods of 8 or 12 days, no infection was obtained when aphids fed on the indicators for 1 day, but that infections occurred when they fed on the indicators for several days. It seemed that this might be due either to the virus having a latent period in the vector or merely to the increased probability of an aphid infecting an indicator on which it fed for a long time as compared with one on which it fed for a short time.

To obtain additional data on this point, further trials were made in 1947 (see Tables 6 and 7). It is thought that the results are inconclusive, but that the most probable explanation is that the virus has a latent period in the vector.

TABLE 6. *Experiment 6*

Infector: F. vesca infected with virus 3.
Infection feeding period: Various.
Indicator: F. vesca. Five aphids per indicator.

		Infection feeding period			
Transfer	Period	4 days	6 days	8 days	15 days
1	24 hr.	0/5	0/5	0/5	1/5
2	24 hr.	0/5	0/5	0/5	3/5
3	48 hr.	0/5	0/5	0/5	4/5
4	48 hr.	0/5	0/5	2/5	3/5

TABLE 7. *Experiment 7*

Infector: F. vesca infected with virus 3.
Infection feeding period: Various.
Indicator: F. vesca. Five aphids per indicator.

		Infection feeding period			
Transfer	Period	4 days	6 days	8 days	12 days
1	24 hr.	0/5	0/5	0/5	0/5
2	24 hr.	0/5	0/5	0/5	0/5
3	48 hr.	0/5	0/5	1/5	1/5
4	96 hr.	0/5	1/5	2/5	3/5

(iv) *Persistence*

Virus 3 persists in the vector for at least 6 days as will be seen from Table 8.

TABLE 8. *Persistence of virus* 3, 1945 (*two trials*)

Infector: Royal Sovereign plant infected with virus 3.
Infection feeding period: 3 weeks or 6 weeks in different trials.
Indicator: F. vesca. Five aphids per indicator.

Transfer	Period	Plants infected
1	48 hr.	6/10
2	48 hr.	3/10
3	48 hr.	2/8
4	48 hr.	2/5

(v) *Combination with other viruses*

The combination of virus 1 and virus 3 in Royal Sovereign by grafting (two grafts, 1945) resulted in the production of symptoms of severe crinkle on all four plants and on runners subsequently taken from them. The leaves were distorted and had chlorotic spots, some of which developed necrotic centres. Symptoms were more severe than those caused by virus 3 alone and were most conspicuous in May and June.

The combination of virus 2 and virus 3 in Royal Sovereign (two grafts) resulted in the production of symptoms of yellow-edge on all of the plants and on runners taken from them. The yellow-edge produced by viruses 2 and 3 in combination was similar to that produced by viruses 1 and 2 but differed in that chlorotic spots (which were particularly prominent in the spring and early summer) could usually be distinguished on the leaves of plants infected with viruses 2 and 3, but not on those infected with 1 and 2.

Royal Sovereign plants infected with virus 1, virus 2 and virus 3 together (two grafts) developed very severe yellow-edge symptoms. The plants were much reduced in vigour and only a very few, short stolons were produced in the year following infection.

The addition, by grafting, of virus 3 to two Huxley strawberry plants already affected with mild crinkle produced no apparent effect.

DISCUSSION AND CONCLUSIONS

Three viruses have now been isolated from complexes occurring in strawberry plants. As the evidence suggests that they are distinct entities they are, therefore, for the time being, referred to as viruses 1, 2 and 3. These provisional designations have no connexion with the strawberry viruses 1, 2 and 3 of James Johnson (Smith, 1937).

From the experiments described it is probable that virus 3 has a latent period in the vector, or that the vector is less efficient in transmitting the virus immediately

after leaving the source of infection than it is some days later. Thus from Tables 6 and 7 it will be seen that, with infection feeding periods of 12 days or less, no infections were obtained during the first 48 hr. on the indicators (i.e. during transfers 1 and 2) and only two infections occurred during the second 48 hr. (transfer 3). During the next 48 hr. or 96 hr. period (transfer 4) eight plants were infected. The distribution of infected plants –(in all of the transfers after 15 days infection feed, in the last two transfers only after 12 and 8 days' infection feed, in the last transfer only after 6 days' and in none of the transfers after 4 days' infection feed) is also in accordance with what would be expected to occur if the virus has a latent period in the vector. The data of Table 5 can be interpreted similarly.

If a latent period is assumed, however, the results shown in Table 3 appear anomalous. In this experiment the symptoms on indicators of the first transfer feed were originally diagnosed as being caused by combined infection with virus 1 and virus 3, but if virus 3 has a latent period this is unlikely. These symptoms, and those on the first transfer indicators in Exp. 4, may, however, have been caused by some other combination of viruses.

It is not possible to assign an exact figure to the duration of the presumed latent period since the minimum infection feeding period for transmission is not known (e.g. transmission might have been effected after a 4-day infection feed if the number and duration of transfer feeding periods had been extended). It appears, however, that the latent period is at least 4 days and may be as much as 10 days, which is much longer than the latent periods previously recorded for other aphis-transmitted viruses (Bawden, 1943, p. 69).

Viruses 1, 2 and 3 are considered to be distinct since they differ in vector relationships, in the incubation period of the diseases which they cause and in the symptoms which they produce on *Fragaria vesca* and Royal Sovereign. These differences are summarized in Tables 9 and 10. It is probable that virus 3 is identical with the persistent virus isolated by previous workers from plants infected with severe crinkle (Wood & Whitehead, 1947).

TABLE 9. *Comparison of viruses* 1, 2 *and* 3

Virus	Minimum infection feeding period required for transmission	Persistence in vector	Period for development of first symptoms on *F. vesca*
1	1 hr.	About 3 hr.	2–3 weeks
2	24 hr.	Several days	4–6 weeks
3	6 days	Several days	4–8 weeks

The symptoms produced on Royal Sovereign by virus 3 show some similarity to those produced by virus 1, but they are much more severe. They are most severe when first produced and become milder after some months. A similar partial recovery and even the complete disappearance of symptoms from severe crinkle plants infected by aphids has been reported by Vaughan (1933) and Hopkins (1941).

After the initial recovery symptoms produced by virus 3, or viruses 1 and 3 together, fluctuate and are most conspicuous in May and June, which accords with field observations of crinkle (Beaumont & Staniland, 1945).

TABLE 10. *Symptoms produced by viruses* 1, 2 *and* 3

Virus or combination	Symptoms on *F. vesca*	Symptoms on Royal Sovereign
1	Chlorotic spotting; leaf distortion	Faint chlorotic spotting
2	Slight chlorotic spotting; slight chlorosis; leaf cupping	Slight chlorosis (mainly at margins of leaves)
3	Severe chlorotic spotting; leaf distortion; vein necrosis	A mild form of severe crinkle (definite chlorotic and necrotic spotting)
1+2	Chlorotic spotting; severe stunting; sometimes death	Severe yellow-edge (chlorosis of leaf margins; stunting)
1+3	—	A mild form of severe crinkle
2+3	—	Severe yellow-edge
1+2+3	—	Very severe yellow-edge

The fact that severe crinkle as found in the field is often more severe than that produced by viruses 1 and 3 together may be due merely to environmental factors. It is considered more likely, however, that yet another virus is involved in the 'crinkle' complex and that this virus may have been responsible also for the apparently anomalous results obtained in transfers from severe crinkle infected plants to Royal Sovereign (Table 3) in which there was no evidence of a latent period.

It has been demonstrated, however, that a form of severe crinkle is caused by a complex of two viruses, virus 1 and virus 3, and as virus 3 has not been obtained from plants infected with mild crinkle it appears that 'severe crinkle' is etiologically distinct from 'mild crinkle'. Such distinction in etiology had been previously deduced only from the symptoms exhibited by infected plants (Zeller, 1933; Harris, 1938).

It had been shown previously that the combination of virus 1 and virus 2 produced symptoms of yellow-edge in Royal Sovereign (Prentice, 1948) and it has now been shown that a second, etiologically distinct, form of yellow-edge can be caused by virus 2 and virus 3. So far no naturally infected plants containing virus 2 and virus 3 alone have been identified, but a virus apparently identical with virus 3 has been isolated from a yellow-edge affected Royal Sovereign plant containing also viruses 1 and 2.

EXPLANATION OF PLATE 2

Fig. 1. *Fragaria vesca* plant infected with virus 3.
Fig. 2. Leaves from *F. vesca* plant infected with virus 3.

Fig. 1.

Fig. 2.

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STUDIES IN THE DIAGNOSIS OF MINERAL DEFICIENCY

V. MANGANESE DEFICIENCY IN WHEAT

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(With 2 Text-figures)

At fourteen sites where winter wheat was growing commercially, twelve of which were known or suspected to be deficient in manganese, a spray of manganese sulphate was applied late in the shooting stage of development, and the effect on yield of grain estimated. Samples of certain organs of unsprayed wheat plants were gathered on three occasions between tillering and ear emergence, and samples of weed leaves on the same occasions and after harvest; these samples were analysed spectrographically for manganese.

The results indicate that analysis of the wheat plant can be used to forecast its response to a manganese sulphate spray, and the following tentative limiting values for manganese content are put forward, above which no increase in grain yield as a result of treatment may be expected: (*a*) during tillering, 34 p.p.m. manganese in dry matter of lower leaf blades; (*b*) just prior to ear emergence, 36 p.p.m. manganese in dry matter of stems. The highest correlation obtained between response and manganese content was that with the values for stem samples gathered just prior to ear emergence.

Analysis of weed samples can only be expected to indicate gross differences in the manganese status of different sites.

Introduction

Although at the beginning of this century investigators in Japan (Asō, 1902, 1904; Nagaoka, 1903, 1904; Loew & Sawa, 1902; Fukutome, 1904) and France (Bertrand, 1905) had reported improvements in growth and yield of crops following manganese treatment, it was not until certain chloroses occurring in the field in America (Gilbert *et al.* 1926; Gilbert & McLean, 1928) and the widespread 'grey speck' disease of oats in Europe and Australia (Samuel & Piper, 1928) were shown to be due to manganese deficiency that this nutrient came to be regarded as of practical importance in agriculture. Since that time manganese deficiency has been identified in many parts of England. Kent may, in fact, claim to be the classic locality for manganese deficiency in Britain, since it was the marshlands of that county which gave the name of 'marsh spot' to the disease of peas now known to be due to manganese deficiency (Heintze, 1938, 1946; Glasscock & Wain, 1940). Consequently when the Kent marshlands were ploughed during the war the expected trouble from manganese deficiency resulted. Grey speck disease of oats appeared and characteristic manganese deficiency symptoms were also seen on potatoes and other crops. Yields of the more susceptible crop plants were often much below expectations.

The crop in which manganese nutrition has been most intensively studied is oats. Not only was this one of the first crops in which manganese deficiency symptoms were clearly identified in the field (Samuel & Piper, 1928), but it is also particularly

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susceptible to deficiency of this element, and develops more clearly defined symptoms than many other species. By contrast, the manganese nutrition of the other small grains has been relatively little studied. It is generally agreed that rye and barley are comparatively resistant to manganese deficiency, wheat being intermediate in susceptibility between these crops and oats (though Gallagher & Walsh, 1943*a*, regard oats and wheat as equally susceptible). Deficiency symptoms have been observed on wheat in the field in Germany (Rademacher, 1935), Ireland (Gallagher & Walsh, 1943*b*), Sweden (Wahlin, 1944), northern England (Plant, 1945) and India (Ramamoorthy & Desai, 1946), and are described as bearing a broad similarity to 'grey speck' of oats, though they are generally less well defined. Increases in yield and improvements in growth of wheat as a result of manganese treatments have not infrequently been reported (Asō, 1902; Leeper, 1935; Gallagher & Walsh, 1943*a*, *b*; Barclay, 1945; Roach & Barclay, 1946). Most of these investigators applied the manganese in the form of a manganous sulphate spray—the method generally found most effective for field crops (Rademacher, 1935; Wallace & Ogilvie, 1942).

There is no need to discuss here at length the use of plant analysis for the diagnosis of mineral deficiency, for the subject has been adequately reviewed elsewhere (Goodall & Gregory, 1947). Among those who have investigated the use of such methods for wheat, the subject of the present investigation, Münter (1919) used samples of grain and straw at maturity, Loehwing (1928) and Maume & Dulac (1934–42) the whole aerial part of the plant before maturity, Holmes, Roberts & Roach (1945) the younger leaves and Ramamoorthy & Desai (1946) the 'flag' leaves. In respect of manganese deficiency in cereals, most of the observations on diagnostic plant analysis relate to oats. Samuel & Piper (1929) gave 14 p.p.m. manganese in the dry matter of the whole plant as the limit below which deficiency symptoms might be expected; later (Piper, 1931; Leeper, 1935) the figure of 15 p.p.m. was quoted. A relationship between manganese content and deficiency symptoms was also found by Gilbert *et al.* (1926), Albert (1932) and Nicholas (1946); Lundegårdh (1931, 1932), Gerretsen (1937) and Steenbjerg (1944), on the other hand, failed to find any consistent relationship between the incidence of grey speck symptoms and the manganese content of the plant. Leeper (1935) claimed that his limiting value of 15 p.p.m. of manganese in dry matter applied to other cereals as well as to oats. Gallagher & Walsh (1943*a*) found that among wheat varieties grown on the same soil the manganese content was lower in those most subject to deficiency—unlike Steenbjerg & Boken (1939) with oats; Holmes *et al.* (1945) reported the use of plant analysis for the diagnosis of manganese deficiency in wheat in Devonshire. Ramamoorthy & Desai (1946) found that, in wheat leaf samples from areas showing deficiency symptoms, the manganese content of the ash ranged from 16·4 to 80·4 p.p.m., whereas the corresponding figures from healthy areas were 35·1–171·8 p.p.m. As examples of the diagnosis of manganese deficiency in other plants may be mentioned the facts that Epstein & Lilleland (1942) quoted 17 p.p.m. as the limiting manganese content for deficiency symptoms in peach leaves, and that

Drosdoff (1943) gave 30 p.p.m. as the corresponding figure for tung; Hale, Watson & Hull (1946) found that the leaves of sugar-beet plants showing symptoms of manganese deficiency almost always had a manganese content less than 25–30 p.p.m. Marsh & Powers (1945) considered 100 p.p.m. as the manganese content below which responses to manganese treatment might be expected in beans and sugar-beet.

It will be noted that most investigations of the analytical diagnosis of manganese deficiency have been concerned only with possible relationships between manganese content and the incidence of deficiency symptoms. From the practical point of view, however, the presence of deficiency symptoms may be irrelevant unless they directly affect the quality of the product, as in marsh spot of peas, or unless it has been shown that they are accompanied by a decrease in yield—which may not always be the case (Roach & Hoblyn, 1945). The practical agriculturist needs an indication of the probable results of a projected treatment in terms of economic return. Accordingly, it is desirable to attempt to correlate plant analysis values with the increases in yield resulting from given treatments—especially as nutrient deficiencies may often cause substantial decreases in yield at levels not severe enough to induce recognizable symptoms. With the exception of the work of Marsh & Powers (1945), no attempts appear to have been made to work out a relationship between yield response to manganese treatment and the manganese content of the plant. The work described below was carried out in the hope of obtaining further information of this type.

The use of plant analysis for the diagnosis of nutritional deficiencies in annual crops has the possible disadvantage that by the time diagnosis is possible it may be too late to apply the necessary corrective treatments—in which case the diagnosis can only be used if relevant to subsequent crops on the same land. This difficulty is of less importance in the case of manganese than of some other nutrients, for it has often been found (Rademacher, 1935; Wallace & Ogilvie, 1942; Gallagher & Walsh, 1941, 1943*a*; Barclay, 1945) that a manganese sulphate spray can be effective up to quite a late stage in the development of cereals. However, it is clear that it would simplify the organization of control measures if the prospect of a deficiency could be forecast before the crop was planted; occasional suggestions (see Goodall & Gregory, 1947) have been made to the effect that analysis of weeds growing on the site before planting-time might enable such a forecast to be made. Very little practical work has been done in this connexion, however, and none on manganese deficiency; it therefore seemed of possible interest to include weed analysis in the investigations about to be described.

EXPERIMENTAL PROCEDURE

In 1943 it was found that winter wheat had been planted at eleven sites (ten in Kent, one in Sussex) previously showing manganese deficiency. To these were added a field on the Experimental Farm of East Malling Research Station suspected of manganese deficiency, and, for purposes of comparison, two fields on the

TABLE 1. *Notes on experimental sites*

Reference letter	Farm, with latitude and longitude	Field	Wheat variety	Preceding crop	Notes
A	Shirley Moor (51° 3′ N., 0° 45′ E.)	O.S. 367	Yeoman	Trefoil	Lower-lying portion*
B	,, ,,	,,	,,	,,	Better drained portion*
C	,, ,,	O.S. 77	,,	Pasture	*
D	,, ,,	O.S. 76	,,	Field beans	*
E	,, ,,	O.S. 1250	Vilmorin	,,	*
F	,, ,,	O.S. 1244	Holdfast	,,	*
G	,, ,,	O.S. 1245	White Victor	,,	Undersown with broad red clover during summer*
H	Catt's Farm (51° 1′ N., 0° 45′ E.)	Designation not known	Squareheads Master	Not known	Low-lying portion*†
I	Priory Farm (51° 2′ N., 0° 46′ E.)	,,	Garton's 60	,,	*
J	,, ,,	,,	Uncertain	,,	*†
K	Novington Farm (50° 55′ N., 0° 4′ W.)	Kemp's Meadow	White Victor	Oats	Previous failures of oats
L	East Malling Research Station (51° 18′ N., 0° 27′ E.)	O.S. 330 (Plot P 15)	Els	Potatoes	‡
M	,, ,,	O.S. 213*d* Deadman Field (Plot 3)	,,	Strawberry runners	No record of manganese deficiency
N	,, ,,	O.S. 91 Merryweather Field (Plot 3)	,,	Potatoes	No record of manganese deficiency

* Grazed by sheep during spring to reduce risk of lodging.

† Positive results from manganous sulphate spray obtained in another part of the field by W. A. Roach (private communication) during 1943.

‡ Adjacent to lake-bed from which manganese deficiency symptoms in numerous species have been reported (Garner & Roach, 1945: Roach & Hoblyn, 1945).

Experimental Farm with no record of manganese deficiency. Notes on these fourteen sites are given in Table 1.

Four plots, each 10 × 5 yd., were laid out at each site: two for treatment, two as controls. Between 3 and 12 May, treated plots received a knapsack spraying with 0·5% manganous sulphate solution at the rate of 100 gal./acre. At this time the plants at all sites were well advanced in the 'shooting' stage of development. No clear-cut deficiency symptoms were observed either before or after treatment.

Yield estimates were made on control and treated plots about 3 days before the field as a whole was harvested, i.e. between 30 July and 10 August. Labour and transport difficulties unfortunately made it impossible to harvest the whole of the experimental area, so sample areas within each plot were harvested. These consisted of twenty 1-yard lengths of drill row taken at random over the plot (except that at site D part of each plot had been trampled by cattle, and these areas were avoided), the mean spacing of the drill rows in the plot being also determined, to permit the conversion of the results to an area basis. At two of the sites (G and K), however, the wheat had lodged badly, making sampling by drill rows impracticable; here it was necessary to harvest two 1-yard squares taken at random in each plot. The ears were gathered, taken to the laboratory, threshed by hand, and the grain from each plot weighed. Straw yields were not recorded.

Samples of plant material for analysis were taken separately from two control plots at each site on two occasions before and one after treatment, i.e. 24 March–5 April, 15–22 April, and 15–21 May. At the first sampling (I) the plants were still in the tillering stage; at the second sampling (II) they had begun to shoot; at the third sampling (III) ear emergence had almost begun. It would have been preferable (Maume & Dulac, 1942) to take samples from the various sites at precisely comparable stages of development, but this was impracticable. For the first set of samples, forty wheat plants were removed from each plot, but on subsequent occasions twenty plants were adequate. The plants were removed at random over the plot, and the same number of plants was at the same time removed from the plots to be treated, to prevent any possible influence on the estimate of yield increase. Samples of the more abundant weed species at each site were collected at the same time from the control plots, and removed from the treated plots, in the same way as the wheat plants themselves. A fourth series of weed samples (IV) was collected from the control plots after harvest, between 25 August and 6 September. The weed species collected included *Agrostis* sp., *Atriplex patula*, *Cerastium vulgatum*, *Chenopodium album*, *Cirsium arvense*, *Poa pratensis*, *Potentilla anserina*, *P. reptans*, *Ranunculus repens*, *Senecio vulgaris*, *Stellaria media*, *Urtica dioica*, *Veronica agrestis* and *Vicia faba* (self-sown from previous crop).

After transport to the laboratory, the following parts of the wheat plants were separated as potentially suitable types of sample for analysis:

(i) the laminae of the lowest leaves still normally green or showing early signs of senescence;

(ii) the sheaths of these older leaves;
(iii) the whole of the youngest emerged leaves;
(iv) the mesocotyls (occasions I and II only);
(v) the stems, without leaf sheaths (occasion III only).

Of the weeds gathered, only the leaves were used. All samples were prepared for analysis in the way described by Roach (1946), except that drying took place in an electric oven at about 80° C.

The samples were analysed spectrographically for manganese by a modification (Goodall, 1948) of the method described by Roach (1946). Since few of the weed species were found at a majority of the sites, it was not considered worth while to analyse the weed samples other than those of thistle, *Cirsium arvense*, and silverweed, *Potentilla anserina*, which occurred respectively at thirteen and ten of the fourteen sites (Table 2).

TABLE 2. *Sites from which weed samples analysed were gathered on different occasions*

Sampling occasion	*Cirsium arvense*	*Potentilla anserina*
I	C, D, E, H, I, M	—
II	A, B, C, D, E, F, H, I, J, M	A, B, D, E, F, G, H, I
III	A, B, C, D, E, F, H, I, J, M, N	A, B, C, D, E, F, G, H, I, J
IV	A, B, C, D, E, F, H, I, J, L, M, N	A, B, C, D, E, F, G, H, I, J

All analyses were performed at least in duplicate, and the figures quoted below are in each case the means of the duplicate samples from the two control plots.

RESULTS

Yields of grain

On account of the low degree of replication, the precision of the yield results was unavoidably low. The means at each site are shown in Table 3:

TABLE 3. *Yields of treated and control plots (cwt. grain/acre)*

Site	Mean yield		Mean percentage increase of treated plots over control, with its standard error
	Control	Treated	
A	20·1	22·5	11·9 ± 9·3
B	27·3	25·0	− 8·6 ± 8·1
C	12·2	14·5	19·2 ± 14·0
D	25·4	27·5	8·5 ± 9·2
E	10·8	11·9	10·1 ± 18·1
F	8·9	10·4	16·9 ± 27·4
G	18·4	19·5	5·7 ± 12·8
H	9·0	10·7	18·9 ± 13·7
I	11·4	7·9	−30·6 ± 24·6
J	28·5	24·1	−15·6 ± 12·9
K	30·2	26·4	−12·7 ± 10·4
L	20·2	21·1	4·2 ± 3·0
M	29·2	33·3	13·8 ± 12·0
N	28·6	28·7	0·2 ± 0·9

It will be seen that none of the increases in yield, taken individually, reaches significance, and, in fact, the whole of the allegedly deficient sites (A–L), taken collectively, show a mean increase due to treatment of only a negligible 2·3%. This is principally due to the large negative values from Priory Farm (I and J), where it was clear that the sites of the trials had been unfortunately chosen (as already stated, other trials in the same field as J during the same season showed substantial increases in yield), and from Novington Farm (site K), where lodging had interfered with the estimation of yield. The Shirley Moor sites (A–G), however, taken collectively, showed the significant mean increase of 9·1%.

Manganese content of wheat samples

The mean manganese content of the various types of wheat sample taken at different times from the control plots at the various sites are given in Table 4:

TABLE 4. *Manganese content of organs of wheat (p.p.m. in dry matter)*

	Old leaf laminae			Old leaf sheaths			Young leaves			Mesocotyls		Stems
Occasion...	I	II	III	I	II	III	I	II	III	I	II	III
Site												
A	39·8	28·5	41·3	48·5	25·0	18·8	35·5	26·3	27·5	21·3	25·5	26·5
B	47·3	33·5	32·3	62·0	56·3	37·0	42·0	29·7	25·3	20·8	31·0	38·5
C	23·0	24·3	64·5	27·3	34·5	28·8	19·0	21·0	17·5	19·0	38·5	16·8
D	41·5	32·0	29·8	49·5	39·5	31·8	40·0	35·3	31·5	15·0	47·3	29·5
E	48·5	23·0	19·5	31·3	18·3	24·5	26·8	24·8	23·8	30·0	28·0	20·0
F	29·0	24·3	27·3	20·5	23·0	15·0	30·3	25·0	22·0	16·3	35·3	21·8
G	44·0	29·5	22·3	34·8	23·8	20·0	45·3	22·8	28·0	27·8	18·5	32·0
H	29·0	32·0	19·3	51·3	33·3	13·5	32·3	24·3	14·8	37·0	20·8	19·8
I	27·0	34·0	43·0	19·8	26·0	27·0	28·0	27·5	18·0	15·3	24·0	25·0
J	44·3	28·5	33·8	189·0	30·5	20·8	30·8	23·8	23·0	10·5	15·0	27·5
K	30·0	27·3	123·5	40·8	26·0	20·0	27·0	29·0	23·5	49·0	24·8	17·8
L	58·3	33·0	33·3	44·5	65·0	39·5	34·8	35·8	32·5	41·5	—	31·0
M	224·8	150·3	57·0	225·3	193·5	142·5	85·0	70·3	35·8	42·0	52·3	76·8
N	337·5	221·3	68·0	237·5	384·8	137·5	102·5	97·3	53·0	27·0	45·3	79·3
Mean, A–L	38·5	29·2	38·3	51·6	33·4	23·1	32·5	27·1	24·0	25·3	28·1	25·5
Mean, M and N	281·1	185·8	62·5	231·4	289·1	140·0	93·8	83·8	44·4	34·5	48·8	78·0

Certain points of some interest emerge directly from these results. It will be noticed that the manganese content in the leaves shows a general tendency to decrease as the season advances, this decline being proportionately more marked in samples where manganese was in ample supply (M and N); the manganese content in the mesocotyls, on the other hand, shows a slight tendency to rise during April. The older leaves (laminae and sheaths) generally contained rather more manganese than the younger, as has also been found in several dicotyledonous species (Bertrand & Rosenblatt, 1922; Anderssen, 1932; Ward, 1942; Goodall, 1943; Liebig *et al.* 1943; Lyon *et al.* 1943; Lagassé, 1943; Drosdoff, 1944); the possibility cannot be eliminated, however, that this simply represents greater liability of the older leaves to contamination, and more difficulty in cleaning them.

These data confirm the distinction made between the sites A–L, from which manganese deficiency had been reported or suspected, and the sites M and N which were considered normal in manganese nutrition. The difference in manganese content between these two groups of sites appears to be most marked in the older leaves and sheaths, especially at the earlier stages of development (cf. Goodall & Gregory, 1947). On the last occasion of sampling, the difference in manganese content between the two groups of sites was rather greater proportionately in the stems and sheaths than in the leaf blades and young leaves.

Relation of yield results to manganese content of wheat

The correlation coefficients between the percentage difference in grain yield of control and treated plots on the one hand, and the manganese content of the various types of wheat samples on the other, were calculated. On account of the aberrant yield results at certain sites, particularly Priory Farm (I and J), the correlations when data from all fourteen sites were used were uniformly low and non-significant. Correlations were also computed using only the data from the seven Shirley Moor sites (A–G); these are shown in Table 5:

TABLE 5. *Correlation coefficients between yield response of wheat to manganese sulphate spray at different sites on Shirley Moor, and manganese content of wheat material*

	Correlation coefficient (r) at occasion of sampling		
Organ analysed	I	II	III
Lower leaf blades	−0·028	−0·784	+0·407
Lower leaf sheaths	−0·675	−0·371	−0·571
Upper leaves	−0·696	−0·471	−0·377
Mesocotyls	−0·234	+0·434	—
Stems	—	—	−0·896

For $P=0{\cdot}05$, $r=0{\cdot}755$.

It will be noted that two of these correlations are significant, namely those for manganese content of the lower leaf blades at the second occasion of sampling, and for that of the stems at the third. The regressions for these two significant correlations are shown graphically in Figs. 1 and 2. It is possible from the regressions to estimate the manganese content values corresponding to zero response, which are:

Lower leaf blades, occasion II ... ... 34 p.p.m.
Stems, occasion III ... ... 36 p.p.m.

Plants with manganese content above these levels might, therefore, be expected not to give an increased yield of grain following manganese treatment.

Manganese content of weed leaves

In Table 6 are given the values for manganese content of the leaves of *Potentilla anserina* and *Cirsium arvense* gathered from the control plots at the various sites.

The manganese content values for thistle leaves are generally rather lower, those

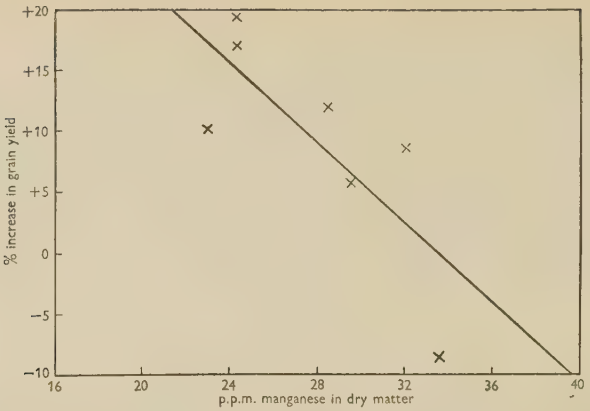

Fig. 1. Regression of percentage increase in yield following manganese sulphate spray on manganese content of lower leaf blades of wheat during tillering.

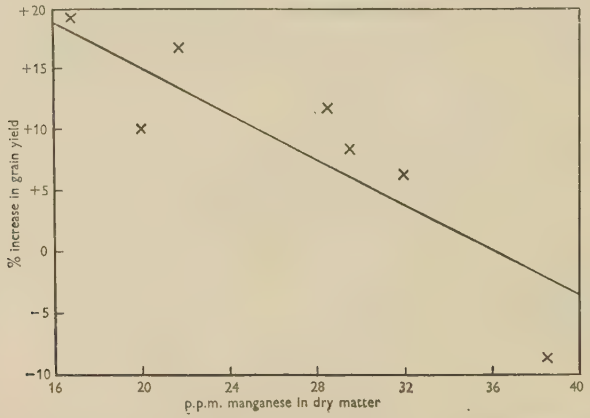

Fig. 2. Regression of percentage increase in yield following manganese sulphate spray on manganese content of wheat stems just prior to ear emergence.

TABLE 6. *Manganese content of weed leaves (p.p.m. in dry matter)*

	Cirsium arvense				*Potentilla anserina*		
Occasion...	I	II	III	IV	II	III	IV
Site							
A	—	22·3	19·5	35·0	31·5	40·8	28·5
B	—	34·3	20·8	23·8	62·5	50·0	34·8
C	23·0	17·5	23·5	23·5	33·5	70·0	31·8
D	31·5	32·5	28·0	76·5	81·5	54·3	130·0
E	22·0	16·3	16·8	18·5	41·3	31·0	26·8
F	—	24·5	41·5	16·8	—	34·8	21·0
G	—	—	—	—	61·0	50·3	24·8
H	30·5	19·8	22·5	30·0	31·8	35·0	37·5
I	24·5	26·0	24·0	15·5	—	43·0	20·0
J	—	31·5	14·5	16·8	45·5	30·5	20·5
K	—	—	—	—	—	—	—
L	—	—	—	46·8	—	—	—
M	109·3	90·3	86·5	108·3	—	—	—
N	—	—	148·5	252·0	—	—	—

for silverweed rather higher, than for wheat sampled simultaneously. No consistent trend through the season is evident in either species.

The differences in manganese content of thistle leaves at 'deficient' and 'normal' sites are as marked as those in wheat at the last sampling, though proportionately rather less than in the older wheat leaves at the earlier occasions of sampling. This suggests that analysis of thistle leaves at any time of year would at least indicate gross differences in the manganese content of the soil available to wheat.

Relation of yield results to manganese content of weed leaves

Correlation coefficients were computed with the manganese content of the weed leaves in the same way as with the wheat analyses. The values obtained for the Shirley Moor sites are shown in Table 7:

TABLE 7. *Correlation coefficients between yield response of wheat to manganese sulphate spray at different sites on Shirley Moor, and manganese content of weed leaves*

	Correlation coefficient (r) at occasion of sampling			
Weed species	I	II	III	IV
Cirsium arvense	−0·535	−0·664	+0·390	−0·056
Potentilla anserina	—	−0·515	+0·080	−0·068

None of these correlation coefficients reaches significance, though those at the second sampling approach it. Accordingly the analysis of weed leaves cannot be regarded as a satisfactory substitute for analysis of the wheat plants themselves for the purpose of diagnosis of manganese deficiency, though it is possible that it might be of some value if it were necessary to assess the manganese status of land for wheat at a time when wheat was not growing on it.

Relation between manganese content of wheat and weeds

It was also considered of some interest to determine the extent to which the manganese content of the leaf blades of wheat was related to that of the weed leaves sampled. Since for this purpose any inadequacies in the estimates of yield response were irrelevant, the data from all sites could be included. The correlation coefficients found are given in Table 8.

It will be seen that, among the deficient sites alone, the manganese content of the weed leaves is not highly correlated with that of the wheat plants; when, however, the very much higher manganese values from sites M and N are included, the correlations become highly significant, even when the plant material samples in question were collected at different times. It is unfortunate that silverweed did not occur at these two sites, so that comparable figures for this species are not available. We may conclude that, while gross differences in amounts of soil manganese available to plants may affect the manganese content of wheat and thistle similarly, the same may not be true of smaller differences in the range bordering on deficiency.

TABLE 8. *Correlation coefficients between manganese content of wheat leaf laminae at different sites, and manganese content of weed leaves*

Weed species	Occasion of sampling, weed leaves	Occasion of sampling, wheat leaves	r (deficient sites)	r (all sites)
Cirsium arvense	I	I	+0·024	+0·986‡
,,	I	Mean, all occasions	−0·336	+0·989‡
,,	II	II	+0·628	+0·971‡
,,	II	Mean, all occasions	+0·395	+0·964‡
,,	III	III	−0·050	+0·658*
,,	III	Mean, all occasions	−0·477	+0·971‡
,,	IV	I	+0·321	+0·945†
,,	IV	II	+0·038	+0·945‡
,,	IV	III	−0·104	+0 620*
,,	IV	Mean, all occasions	+0·270	+0·945‡
Potentilla anserina	II	II	+0·513	—
,,	II	Mean, all occasions	+0·188	—
,,	III	III	+0·666*	—
,,	III	Mean, all occasions	+0·545	—
,,	IV	I	+0·143	—
,,	IV	II	+0·297	—
,,	IV	III	−0·089	—
,,	IV	Mean, all occasions	+0·112	—

* Significant ($P<0·05$). † Highly significant ($P<0·01$).
‡ Very highly significant ($P<0·001$).

DISCUSSION

Although the value of this work has been reduced by the unfortunate, though unavoidable, lack of precision in the yield results, it confirms the general usefulness of the analysis of plant material as a method of diagnosis of mineral deficiency, and provides an addition to the very limited number of investigations in which an attempt has been made quantitatively to relate the response of a plant to fertilizer treatment with its nutrient content (Crowther, 1937; Craig, 1940; Lundegårdh, 1941; Goodall, 1948). Manganese nutrition is perhaps an unusually convenient subject to treat in this way, since deficiency treatment can be effectively performed at a late stage of development, leaving plenty of time during which diagnosis can profitably be undertaken. Of the organs tested, the mesocotyl appears to be quite unpromising, and indeed would be inconvenient to use in practice; of the other organs, the tentative conclusion is that while the older leaves may provide suitable samples for analysis during tillering, at a later stage of development the stems may be preferable. It is unfortunate, in view of the high correlation observed thereafter, that stem samples were not gathered before mid-May, when the treatment was carried out. However, on two of the fields involved, Roach obtained positive responses to spray treatment on 19 May, ten days later than in the present work, so there seems little doubt that stem and/or sheath samples gathered at a late shooting stage, if analysed without delay, could in fact provide information of practical value on the desirability of spray treatment in the field.

The limiting values quoted for manganese content of these organs, below which response to manganese treatment may be expected, should be regarded as no more than rough approximations, and not necessarily applicable to conditions other than those under which the experiments were performed. They are considerably above the values quoted by other workers (Samuel & Piper, 1929; Piper, 1931; Leeper, 1935): 14–15 p.p.m. in the whole plant for cereals, 17 p.p.m. for peach leaves (Epstein & Lilleland, 1942) and 30 p.p.m. for tung leaves (Drosdoff, 1943); these limiting values apply, however, not to yield response but to the appearance of deficiency symptoms, which were not observed in the present work. The limiting values suggested here are well below the figure of 100 p.p.m. of manganese in the leaf dry matter considered by Marsh & Powers (1945) to be the level below which responses to manganese treatment might be expected in beans and sugar-beet.

Weed analysis appears unpromising—and, where treatment can conveniently be applied to a growing crop, there is little need to employ it. The analysis of thistle leaves, at least, can provide evidence of gross differences in the manganese status of sites, but there is no reason to suppose that it could indicate with any precision the probable response of wheat to manganese spray treatment. Where a wide range of manganese status is covered, the values for manganese content of leaves of thistle and wheat growing on the same site are highly correlated, but this does not apply over a more limited range.

The author's thanks are due to Professor F. G. Gregory, Dr R. G. Hatton and Dr W. A. Roach for their interest in the progress of these investigations; to the Kent War Agricultural Executive Committee and its farm manager Mr J. Kerr; to Mr E. C. Brignall, to Mr D. B. Cooke and to Mr J. K. Harmer, on whose land the experiments were performed; to Mrs P. W. Rose and Mr J. H. Lavender, who helped to obtain the yield estimates; and to Miss E. M. Ford and Miss B. Kidwell, who, with Mr Lavender, assisted in the analyses. Much of the spectrographic apparatus used for the analyses was purchased with the aid of funds made available by the Government Grants Committee of the Royal Society to Dr W. A. Roach.

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FACTORS AFFECTING THE ACTIVITY OF ALATAE OF THE APHIDS *MYZUS PERSICAE* (SULZER) AND *BREVICORYNE BRASSICAE* (L.)

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(With 20 Text-figures)

Apparatus was designed for testing the frequency of flights of aphids under different conditions of temperature, relative humidity, light and pressure. Young aphids (1–4 days after metamorphosis) flew more often and showed less individual variability than older ones; with all ages activity increased for the first few hours under experimental conditions. Starving increased activity for the first 1–2 hr. Aphids used in experiments on a second day flew less frequently than controls which had remained on the plant the first day. Alate *B. brassicae* were more active than *M. persicae*: both species showed alternating periods of activity and quiescence.

At light intensities between 100 and 1000 f.c. there was little difference in flight frequency, but below 100 f.c. activity declined rapidly and apparently ceased with darkness.

Changes in relative humidity temporarily affected flight frequency, a change to a higher humidity retarding, a change to a lower increasing it. After adjusting to the change aphids flew readily at all humidities tested between 50 and 100% with temperatures below 80° F. (26·7° C.). The combination of high humidity and high temperature (90° F. = 32·2° C.) sometimes inhibited flight.

Changes of pressure often increased activity temporarily and flight frequency was greater under fluctuating pressure than under constant pressure.

It is concluded that changes in microclimate in crops are adequate to influence frequency of flight of aphids and consequently the spread of virus diseases.

Davies (1935) suggested that large differences in aphid populations between west and east Wales were the result of the high humidity in the west inhibiting aphid flight. Davies made laboratory experiments on the effect of variation in temperature and relative humidity upon the flight of *M. periscae* (Sulz.) and concluded that over 55° F. (12·8° C.) relative humidities of 70% and above would greatly reduce flight. At 70° F. (21·1° C.) flight activity was progressively reduced as the relative humidity rose from 25 to 100%. Contrary to these statements, Freeman (1945) in studying the distribution of insects by aerial currents, found that Aphididae were most numerous in the aerial fauna under conditions of high humidity. Moericke (1941) stated that high atmospheric humidity does not hinder the take-off of *M. persicae* from potato plants in the field, and Broadbent (1946) caught *M. persicae* and other aphids flying in a potato field even during periods of continuous drizzle.

As alate *M. persicae* play an important role in the dissemination of potato virus diseases (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948) and, no doubt,

of the many other virus diseases of which this species is a vector, it is necessary that the factors affecting flight should be studied in detail. In view of the conflicting reports of the effect of different relative humidities, laboratory studies similar to those of Davies were made. Other flight-influencing factors were studied at the same time, and *Brevicoryne brassicae* (L.) was included in many tests so that two economically important species of different genera could be compared.

Methods

The apparatus consisted of a wooden cabinet with a ground-glass top; in one side was a movable window, fitting closely and sealing the cabinet. Temperature was controlled with heating element, fan and thermostat. Ice was used to obtain temperatures below that of the room. In some experiments a 500 W. filament bulb was suspended above the cabinet, the heat from it being absorbed by a 1-in. layer of flowing water in a large glass tank. The light given was 1000 f.c., measured by a G.E.C. illumination meter. Dimmer lights of similar quality were obtained with bulbs of 40–150 W. The current was kept constant by means of a constant voltage transformer. In other experiments two 80 W. fluorescent lamps were used and no cooling device was required. These gave a light of 500 f.c.; lower illuminations were obtained by raising the lights and blacking-out part of the bulbs. The wave-length of the light approximated to that of daylight and was of equal quality at each level of illumination.

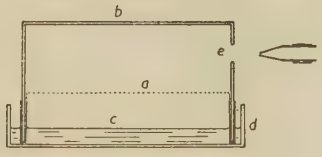

Fig. 1. Apparatus for testing the activity of alate aphids (for description see text).

The aphids were confined above fine copper gauze (Fig. 1, *a*) made of 0·2 mm. wire spaced 0·5 mm. apart, held inside an inverted glass crystallizing dish (Fig. 1, *b*), 3·5 in. in diameter and 2 in. deep, by being sprung against the sides of the dish. A coating of paraffin wax around the inner and outer edges of the dish to a depth of $\frac{3}{4}$ in. kept the gauze in position and prevented the liquid (Fig. 1, *c*) from seeping up the sides of the dish. This liquid, used to control the relative humidity within the vessel, was contained in half a Petri dish (Fig. 1, *d*). A hole (Fig. 1, *e*) bored in the crystallizing dish above the gauze allowed this dish to be lowered into the Petri dish without increasing the pressure within; the aphids were introduced through this hole which was then closed with a short thermometer whose bulb projected into the vessel or with a short length of open glass tubing. I am indebted to Dr R. K. Schofield for the information that the diffusion rate of gases within the vessel would be rapid and that 2 min. after the vessel had been placed over the fluid the percentage

difference between the relative humidities of the air already in the vessel and that immediately above the fluid would be about 1% only. This vessel was placed inside the cabinet and the activity of the aphids was measured by observing the number of times flight was attempted. It was not always possible to distinguish between flight take-offs and 'drops', but since both increased under similar conditions both were counted and recorded as 'flights'. Activity is recorded in the figures, tables and text as the average period (seconds) between take-offs per aphid, no matter how many aphids a batch contained. The time taken for a given number of flights was measured. Unless otherwise stated, the experiments were carried out at 75° F. (23·9° C.) and 50% R.H. (relative humidity). The liquids used were kept within the cabinet so that there should not be temperature differences when changes were made. The same liquids were used at all temperatures, the variations in relative humidity with temperatures over the range tested being slight. With this apparatus the same aphids could be studied under different conditions of temperature, relative humidity and light.

The effect of the solution used to obtain a given relative humidity

It was possible that the increased activity shown by the aphids in Davies's experiments at lower humidities might have been the result of the increasing concentration of sulphuric acid. Preliminary experiments comparing the activity of the same insects over sulphuric acid, potassium hydroxide and various salts at concentrations to give relative humidities of 50, 75 and 90% (Buxton & Mellanby, 1934; Sweetman, 1933) showed that sulphuric acid had no apparent affect on the activity of *M. persicae* at these concentrations. 'Analar' sulphuric acid was used, therefore, throughout the experiments, a small amount of potassium permanganate being added to oxidize any impurities which might be present.

The age factor

For preliminary experiments the aphids were obtained by the method described by Davies, i.e. from the windows of an insectary in which they were bred, usually on turnip, but sometimes on radish and tobacco. Their ability to fly was further tested by freeing them at the bottom of a 4 ft. high dark cylinder which had a 3-in. hole in the top. Over this was placed a glass crystallizing dish in which the aphids, flying to the light, collected.

Tests showed that the batches of alate *M. persicae* used in the experiments varied considerably. In one experiment two batches of twenty aphids were used. About one hundred aphids were placed at the base of the tower and the first twenty to collect in the vessel at the top were called Batch A, the second twenty Batch B. Having been kept during this time (3 hr.) at 65° F. (18·3° C.) and 58% R.H. they were placed in the cabinet at 70° F. (21·1° C.) and 50% R.H. Counts of the time taken for one hundred flights were made after 5, 20, 30 and 40 min.; Batch A was

then changed to 90% and Batch B to 75% R.H. and counts of one hundred flights were made after 5, 15, 25 and 35 min. They were then changed back to 50% R.H. and counts of one hundred flights again made after 5, 15, 25 and 35 min. The results are shown in Fig. 2. Throughout the experiment Batch A was more active than Batch B. Further experiments with aphids of known age were made in an attempt to eliminate one of the factors causing variability.

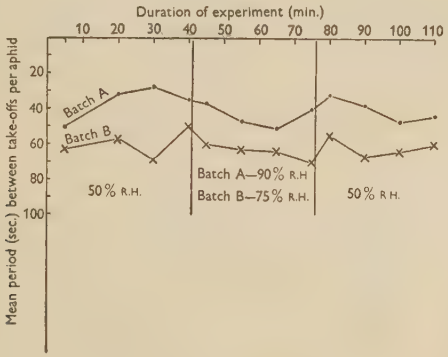

Fig. 2. Difference in activity between two batches of *M. persicae*.

Aphids whose ages since metamorphosis were approximately known were obtained by placing nymphs of alatae on plants under cages and removing to other plants the alatae which emerged each morning. In early experiments the same aphids were tested every day, being returned to the plants after each test. The aphids, however, may be damaged by frequent handling, and although some aphids were used twice in later experiments most were kept on the plants until they reached the age required for testing.

To obtain an estimate of the length of life of alate aphids a number of *M. persicae* and *B. brassicae* were confined on radish plants under glass chimneys. The number of nymphs born each day and the number of alatae remaining alive were noted (Table 1). The *M. persicae* and *B. brassicae* kept over the same period gave birth to 13·0 and 12·5 nymphs per alata (average) respectively. Another batch of *M. persicae* born a week later gave birth to an average of 21·7 nymphs per alata. In each batch more nymphs were born in the first 3 days than in any succeeding period of 3 days; less than half the alatae remained alive after 9 days, although a few lived for 3 weeks. Headlee (1914) and Smith (1937), working with apterous *Toxoptera graminum* Rond. and *Hyalopterus pruni* Geoff. respectively, showed that the length of life and rate of production of young varied with the temperature, the higher the temperature the shorter the life of the aphids and the fewer the young born. The mean temperature of the glasshouse during the first week of the life of Batch A was 3–4° F. higher than the similar period in the life of Batch B, which supports the findings of Headlee and

Smith. The aphids for all the experiments were reared in a heated glasshouse whose temperature fluctuated between 50–80° F. (10–27° C.), being usually about 68° F. (20° C.).

TABLE 1. *Nymphs born of alatae in the glasshouse*

	M. persicae			*B. brassicae*			*M. persicae*			
Date (1948)	No. of alatae	No. of nymphs	Nymphs per alata	No. of alatae	No. of nymphs	Nymphs per alata	Date (1948)	No. of alatae	No. of nymphs	Nymphs per alata
19 Mar.	17	—	—	17	—	—	24 Mar.	17	—	—
20	17	21	1·24	17	6	0·35	25	17	37	2·18
21	17	41	2·41	17	38	2·24	26	17	26	1·53
22	14	18	1·29	15	22	1·47	27	15	28	1·87
23	13	16	1·23	10	13	1·30	28	12	11	0·92
24	13	10	0·77	7	3	0·43	29	11	17	1·55
25	11	8	0·73	7	9	1·29	30	10	13	1·30
26	9	11	1·22	7	6	0·86	31	9	10	1·11
27	9	3	0·33	7	9	1·29	1 Apr.	9	8	0·89
28	7	5	0·71	7	2	0·29	2	8	11	1·38
29	6	3	0·50	6	3	0·50	3	7	9	1·29
30	5	3	0·60	6	3	0·50	4	7	6	0·86
31	5	5	1·00	5	5	1·00	5	6	7	1·17
1 Apr.	1	1	1·00	5	3	0·60	6	6	6	1·00
2	1	0	—	4	0	0	7	5	3	0·60
3	1	0	—	3	1	0·33	8	4	6	1·50
4	1	0	—	3	0	—	9	4	2	0·50
5	1	0	—	3	0	—	10	3	3	1·00
6	1	0	—	3	0	—	11	2	2	1·00
7	1	0	—	3	0	—	12	2	0	—
8	1	0	—	3	0	—	13	2	0	—
9	1	0	—	2	0	—	14	2	0	—
10	0	0	—	2	0	—	15	1	0	—
11	0	0	—	1	0	—	16	0	0	—
Total nymphs per alata			13·03			12·45				21·65

In Table 2 are the results of two experiments in which *M. persicae* of known age were tested daily for a number of days, being returned to the plants after each test. Four counts of one hundred flights were made at intervals of about 30 min., when the aphids were sufficiently active. In these experiments the aphids were most active when 1–4 days old.

TABLE 2. *The activity of alate* M. persicae *on successive days of their lives*

Age of aphids (days)	0–2	1–3	2–4	3–5	4–6	5–7	6–8
1. No. of aphids	13	13	13	9	8	7	6
Mean period (sec.) between take-offs per aphid	87	38	103	83	95	351	812
2. No. of aphids	16	16	12	9	—	8	—
Mean period (sec.) between take-offs per aphid	394	75	49	111	—	817	—

Variation in activity was great at all ages. Aphids became active about 18 hr. after metamorphosis when kept in the experimental chamber. The activity of aphids removed straight from the plant to the experimental chamber showed the greatest variation during the first few hours, during which time activity increased in aphids of all ages. Thus in an experiment with *B. brassicae*, alatae of four ages were placed in the chamber and tested after $\frac{1}{2}$, 3 and $5\frac{1}{2}$ hr. (Table 3).

TABLE 3. *The activity of* B. brassicae *of different ages*

Tested after (hr.)	$\frac{1}{2}$	3	$5\frac{1}{2}$
12 aphids aged 2–3 days	29	15	33 sec. (mean period between take-offs per aphid)
12 ,, 4–5 ,,	23	20	38 ,, ,, ,, ,,
5 ,, 6–7 ,,	87	31	50 ,, ,, ,, ,,
11 ,, 7–9 ,,	69	55	101 ,, ,, ,, ,,

Progressively increasing activity was shown by two batches of *M. persicae* flying under the same conditions as in the previous experiment (Table 4).

TABLE 4. *The activity of 2–3 days old* M. persicae

Tested after (hr.)	1	2	$2\frac{1}{2}$	7	8
Batch A, nine aphids	91	52	50	—	34
Batch B, eight aphids	202	—	81	28	—

In most experiments the activity of *M. persicae* increased at varying rates, but usually rapidly, during the first few hours within the chamber. *B. brassicae* showed this phenomenon to a less degree. Such a phenomenon might be caused by many factors, some of which were eliminated in an experiment in which three batches of 1–2-day-old *M. persicae* were left in the dark for 5 hr., one batch at 75° F. (23·9° C.) and 50% R.H., another at 75° F. and 100% R.H. and the third at 60° F. (15·6° C.) and 50% R.H. When the light was switched on the last batch was put in the chamber at 75° F. and all were tested at intervals during the next 5 hr. (Fig. 3). The increase in activity occurred in all three batches, but was greatest in the one that had been at 60° F. The other aphids, however, presumably had become accustomed to their surroundings as regards temperature and humidity in the 5 hr. of darkness. The only new factors were those of light, and movement stimulated by light. Possibly some reserve of food is slowly used up with movement and this results in a gradual increase in activity: it is shown below that aphids which had recently fed were less active at the start than those which had been starved.

Fig. 4 shows the results of an experiment with *B. brassicae* in which four batches of fifteen aphids, respectively 1–2, 2–3, 3–4 and 4–5 days old were tested at intervals over a period of 10 hr. The older aphids were more active at first, but the younger remained active longer. The results of a similar experiment with *M. persicae* are shown in Fig. 5; in another experiment (Fig. 6), the younger aphids remained more active throughout.

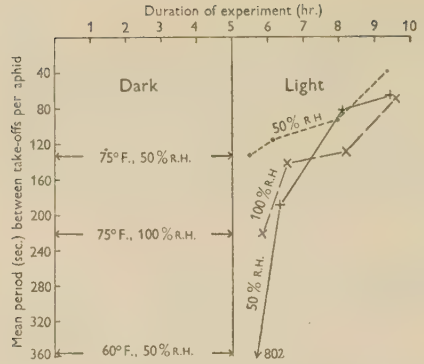

Fig. 3. The activity of *M. persicae* after 5 hr. in the dark under different conditions.

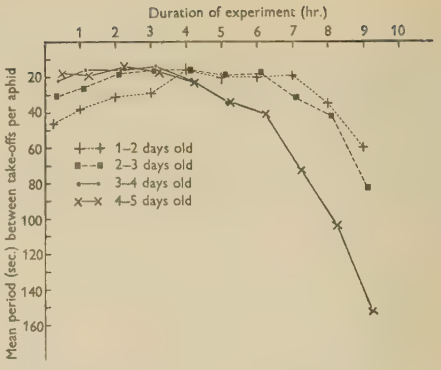

Fig. 4. The activity of *B. brassicae* at different ages.

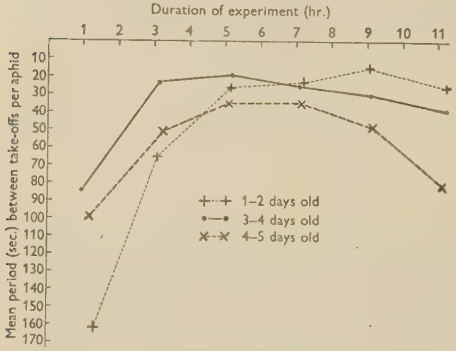

Fig. 5. The activity of *M. persicae* at different ages.

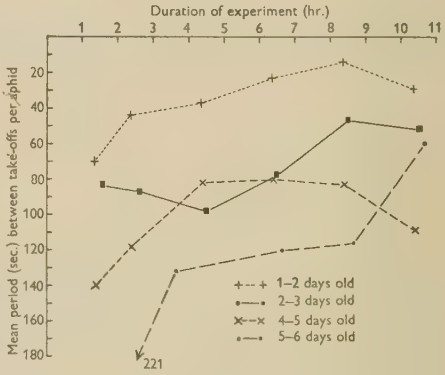

Fig. 6. The activity of *M. persicae* at different ages.

TABLE 5. *The activity of alate* M. persicae *and* B. brassicae *of different ages*

(The number of aphids used in each test is shown in brackets)

Age in days...	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8
M. persicae:								
Mean period (sec.)	86 (13)	21 (11)	33 (14)	59 (6)	49 (10)	83 (9)	245 (6)	59 (7)
between take-offs	88 (13)	32 (13)	44 (12)	76 (9)	95 (8)	89 (7)	1085 (3)	812 (6)
per aphid	394 (15)	43 (13)	45 (6)	95 (9)	235 (5)	115 (7)	—	—
	622 (25)	56 (11)	52 (10)	111 (9)	354 (7)	162 (8)	—	—
	990 (8)	61 (7)	54 (12)	140 (11)	434 (4)	307 (8)	—	—
	1363 (26)	68 (16)	64 (9)	190 (15)	453 (8)	351 (7)	—	—
	—	81 (16)	81 (15)	205 (8)	—	351 (7)	—	—
	—	81 (16)	91 (9)	—	—	817 (8)	—	—
	—	83 (13)	103 (13)	—	—	—	—	—
B. brassicae:								
Mean period (sec.)	75 (39)	21 (10)	12 (4)	16 (15)	19 (15)	23 (13)	82 (11)	69 (11)
between take-offs	—	23 (16)	13 (13)	27 (12)	23 (12)	39 (11)	87 (5)	125 (10)
per aphid	—	28 (11)	19 (13)	28 (14)	30 (9)	47 (4)	181 (7)	—
	—	35 (15)	25 (12)	35 (9)	35 (9)	62 (10)	—	—
	—	36 (13)	26 (15)	40 (20)	36 (17)	63 (13)	—	—
	—	—	29 (12)	—	47 (7)	—	—	—
	—	—	—	—	140 (8)	—	—	—

These and other experiments (Table 5) have shown that aphids 1–4 days old are generally most active. There was often a high death-rate among older aphids during the experiments, and in some the apparent decrease in activity in the older aphids resulted from the death of some individuals, those remaining alive being as active as younger aphids.

Previous activity

Aphids which had been previously exercised were often less active than others which had not. Half a batch of *B. brassicae* was exercised in the cabinet for 3 hr. when 1–2 days old and 5 hr. when 3–4 days old, then tested when 4–5 days old together with the other half of the batch which had remained on the plants the whole time (those on the plants were not completely inactive, since between periods of feeding they could fly within the covering chimney). For the first 5 hr. the previously exercised aphids were less active than the others, after which there was little difference between them (Fig. 7). In a similar experiment twenty-three *M. persicae* were exercised for $10\frac{1}{2}$ hr. when 1–2 days old. Fifteen were alive at the end and were put on a plant to feed overnight. The next morning (14 hr. later) eleven were alive and these were compared with eighteen of the same age which had not previously been exercised; they were only half as active (Fig. 7).

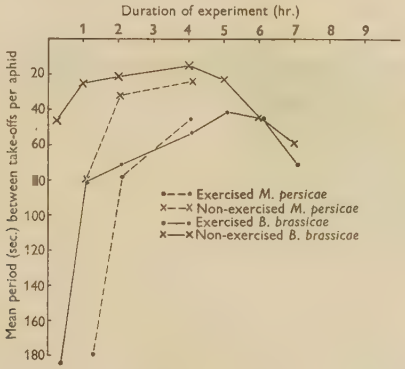

Fig. 7. The activity of previously exercised aphids compared with aphids of the same age not previously exercised.

Starvation

It was not possible to ensure that aphids confined on plants under the chimneys had been feeding a short time prior to testing; indeed, many were taken from the glass wall of the chimney, whereas others were feeding when disturbed. Since the length of time during which the aphids had been starved was likely to affect activity and might account in part for the variability shown by different individuals of the same age, a number of experiments were made comparing aphids which had been starved for a known period (at least) with aphids which had been confined on the plant

(though with no guarantee that they had been feeding). When removing the aphids to be starved care was taken not to disturb any in the act of feeding, and where possible specimens were taken from the chimney glass. The starved aphids were

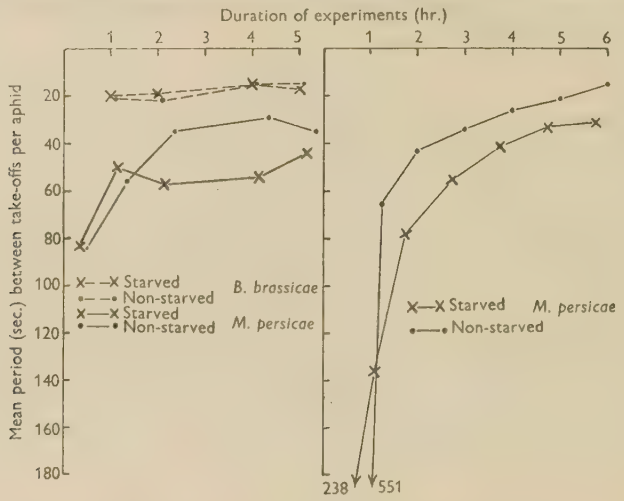

Fig. 8. Fig. 9.

Fig. 8. The activity of starved and non-starved aphids of the same age.

Fig. 9. The activity of starved and non-starved *M. persicae* at the same age.

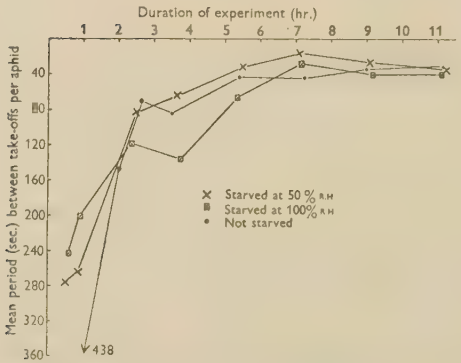
Duration of experiment (hr.)
1 2 3 4 5 6 7 8 9 10 11
Mean period (sec.) between take-offs per aphid
40 80 120 160 200 240 280 320 360
× Starved at 50 % R H
■ Starved at 100% R H
• Not starved
438

Fig. 10. The activity of *M. persicae* starved at different relative humidities and not starved.

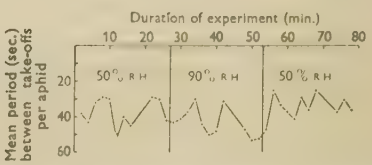

Fig. 11. Periodicity shown by twenty *M. persicae* (counts of twenty flights at 2 min. intervals).

confined in the test capsules over soil under similar conditions of temperature and humidity to those feeding. Figs. 8 and 9 show the results of two such experiments. There was little difference between starved and non-starved *B. brassicae*; in both

experiments the non-starved *M. persicae* were less active at the beginning, but soon flew more frequently than the starved aphids.

In another experiment one-third of a batch of 1–2-day-old *M. persicae* were starved for 12 hr. overnight in a capsule at 50% R.H., one-third at 100% R.H. and one-third were left on the plant. All were kept in the glasshouse at the same temperature and then were tested at 75° F. (23·9° C.) and 50% R.H. with the results shown in Fig. 10. Again the starved aphids were more active than the others in the first 2 hr., but after that there was little difference between them.

Thus recent feeding or starvation affected the activity of the aphids, but did not entirely account for the marked increase in activity invariably shown in the first 2 hr. within the capsule.

Periodicity in flight activity

There was some evidence of periodicity in the activity of the aphids (e.g. Fig. 11). In one experiment twenty *M. persicae* were kept for 2 hr. in the cabinet and then the time for two hundred flights was taken, being recorded after each ten flights

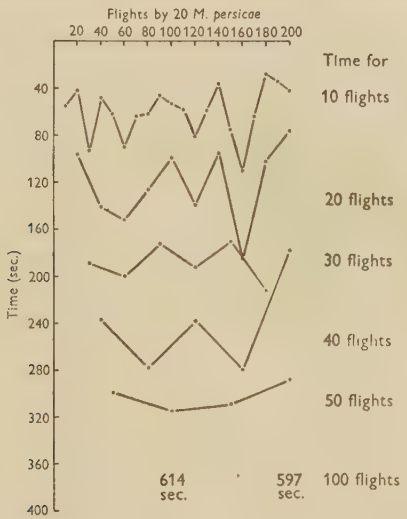

Fig. 12. Different multiples of the time for ten flights by twenty *M. persicae*.

(Fig. 12). It was concluded that reasonably comparable results would be obtained if the number of flights counted was 3–5 times the number of aphids. In this instance, with twenty aphids, the figures were:

2½ times, i.e. fifty flights	5 times, i.e. one hundred flights
299 sec.	614 sec.
315	597
309	—
288	—

In an experiment with ten *M. persicae* three 5 min. counts were made, the number of take-offs per sec. being recorded on squared paper; the time was given by a metronome ticking once per sec. The numbers of flights were:

Run 1, after	45 min.	99
,, 2, ,,	85	105
,, 3, ,,	125	125

The number of flights/5 sec. period was counted (Table 6).

TABLE 6. *Flight frequency in a batch of ten* M. persicae

	Run			Total	
Flights/5 sec. period	1	2	3	Observed	Expected
0	9	16	4	29	28·9
1	25	11	17	53	52·9
2	10	18	18	46	48·4
3	11	10	12	33	29·5
4	4	3	5	12	13·5
5	1	2	2	5	4·9
6	0	1	1	2	1·5

These data show that the frequency of the observed number of flights was very similar to that which would have been expected had the flights been entirely at random and independent of each other. The periodicity shown when a number of aphids are confined together is probably the result of the coincidence of periods of activity and quiescence of several aphids. That individual aphids vary greatly in their activity was shown by two 1–2-day-old *M. persicae* which were tested in separate capsules, under the same conditions as above, for 15 min. after 90 min. under these conditions (Table 7). Aphid no. 1 had a burst of activity in the middle

TABLE 7. *Flights/10 sec. period by individual* M. persicae

	Aphid	
Flights/10 sec. period	1	2
0	52	46
1	26	40
2	8	4
3	3	0
4	1	0

of the period, with long rests before and after, whereas no. 2 flew at frequent intervals throughout the period.

In a third experiment (Table 8) a single aphid was watched as before, but the duration of each flight was recorded for 30 min. (the aphid had been in the chamber for 30 min. before the records were made). Most of the flights were of short duration, of 1 sec. or less, and the intervals between flights were short, apart from three rests of over a minute. The longest period of continuous flight was 11 sec., but on another occasion a 1–2-day-old *M. persicae* flew for 11 min. 8 sec. It is not suggested that results obtained in the small experimental chamber are related to the frequency of flight in the field.

TABLE 8. *Duration of flights by one* M. persicae

	No. of flights	Intervals between flights	No. of flights
Drops	29	0–10 sec.	55
Under 1 sec.	45	11–20	32
1	19	21–30	11
2	8	31–60	6
3	2	61–100	1
4	2	101–130	2
5	1		
10	1		
11	1		

DIFFERENCES BETWEEN *M. PERSICAE* AND *B. BRASSICAE*

At all ages alate *B. brassicae* were more active than *M. persicae* (Table 5, Figs. 7, 8), whereas the apterae of *B. brassicae* are much more sluggish than those of *M. persicae*. Both apterae and alatae of *B. brassicae* took a long time to withdraw their mouthparts when disturbed while feeding; *M. periscae* withdrew rapidly. An obvious difference between the two species in the flight capsules was that *M. persicae* spent most of the time on the underside of the upper glass surface, nearest to the light, but *B. brassicae* spent most time on the edge of the gauze trying to climb up the glass wall and only rarely succeeding. Evidently the small membranous pads between the claws and at the base of the tarsus (Davies, 1936) are not as efficient for gripping glass in *B. brassicae* as in *M. persicae*.

LIGHT INTENSITY

Little information is available about the effect of different light intensities upon the activity of aphids. It has often been observed that alatae orientate themselves towards the brightest light before taking off, e.g. towards the sun outside, or the window indoors.

An experiment with *M. persicae* showed that they probably do not fly in the dark. After $2\frac{1}{2}$ hr. in the experimental chamber a batch of 2–3-day-old aphids was flying at the rate of one flight by one aphid every 50 sec. They were then left in darkness for $4\frac{1}{2}$ hr. When the light was turned on again every aphid was still; continuous records of two hundred flights were then made, the first hundred having an average period of 63 sec. between take-offs per aphid (i.e. $11\frac{1}{2}$ min.), the second hundred an average of 44 sec. (i.e. 8 min.). One hour later the average was 34 sec. In a similar experiment twenty *B. brassicae* were flying with mean periods of 47 sec. between take-offs per aphid in a light of 100 f.c. They were then left in the dark for $1\frac{1}{4}$ hr.; when the light was switched on again all were still and there was no movement for 1 min. Then crawling began and flying after 2 min. After 5 min. they were tested and flew with mean periods of 83 sec. between take-offs per aphid. After about 14 min. they had returned to their former activity.

Experiments were made on the effect of different light intensities on the activity of *M. persicae*, *B. brassicae* and *Hyperomyzus staphyleae* (Koch) using 500 W. to 40 W. filament electric bulbs to give light intensities within the cabinet of 1000 to 10 f.c. The time under each light before readings were taken varied from 5 to 30 min.

in different experiments. The three species behaved similarly and results from seven experiments are given in Fig. 13, activity at 1000 f.c. being regarded as standard. There was a slight decrease in activity from 1000 to 100 f.c.; then activity fell quickly until at 30 f.c. the aphids were only half as active as at 1000 f.c. In an experiment under fluorescent lamps observations were made on two batches of *M. persicae* and one of *B. brassicae* under lights ranging from 1 to 500 f.c. After 2 hr. at 500 f.c. the light was dimmed to 100, then to 50, 30 and 10 f.c., activity being tested 15–30 min. after the changes. The light was then reduced to 1 f.c., which was too dark for counts to be made. *M. persicae*, however, were seen to be crawling and some flights took place. The *B. brassicae* were almost still, though one flight was observed. Frequent flying by *M. persicae* took place at 2 f.c.; the light was then raised through 10, 100 to 500 f.c., lowered through 100 to 10, and finally raised through 100 to 500 f.c. again. These results are shown in Fig. 13; they are similar to

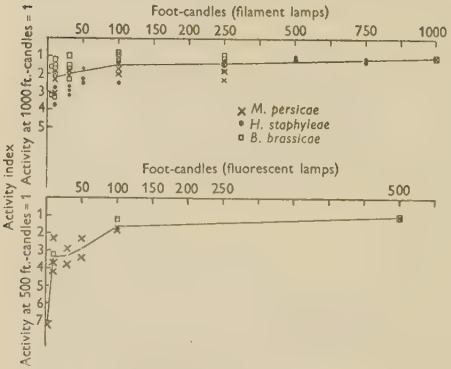
Foot-candles (filament lamps)
50 100 150 200 250 500 750 1000
Activity index
Activity at 1000 ft.-candles = 1
Activity at 500 ft.-candles = 1
× M. persicae
• H. staphyleae
□ B. brassicae
Foot-candles (fluorescent lamps)
50 100 150 200 250 500

Fig. 13. The activity of aphids at different light intensities.

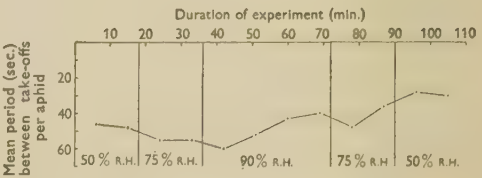

Fig. 14. The activity of *M. persicae* at different relative humidities.

those obtained under filament lamps, except that below 100 f.c. the decline in activity was greater. Whereas about 65% of the luminosity of filament lamps is emitted in the yellow-red region of the spectrum, only about 45% of the light emitted by fluorescent lamps lies in this range. It may be that aphids are stimulated to greater movement at low light intensities by yellow-red than by blue-green light.

In the experiments all the aphids flew actively at light intensities between 10 and 1000 f.c. At low intensities the previous conditions are apparently important: thus *B. brassicae* behaved very differently at 10 f.c. according to whether they had previously been at a higher or lower intensity, as follows:

	Mean period (sec.) between take-offs per aphid
At 10 f.c. following gradual reduction from 500 f.c.	20
At 10 f.c., 5 min. after increase from 1 and 2 f.c.	150
At 10 f.c. 20 min. after increase from 2 f.c.	50

Relative humidity

Experiments on the effect of relative humidity on aphid flight showed that whereas changes affected flight frequency (a change to a higher humidity often retarding activity for a short time and a change to a lower often increasing activity) aphids accommodated themselves to the particular humidity. In one experiment twenty *M. persicae* were placed in the experimental chamber at 73° F. (22·8° C.) and 50% R.H. after being kept at 64° F. (17·8° C.) and 55% R.H. for 3 hr. Counts of flights were made as shown in Fig. 14, the crystallizing dish being moved from 50 to 75 to 90 to 75 to 50% relative humidities. The aphids were allowed 6 min. over the new humidity after each change before the first count was made. Flight frequency at the three humidities was:

R.H. (%)	Mean period (sec.) between take-offs per aphid
50	38
75	49
90	49

In a similar experiment (Fig. 2) flight frequency at the different humidities was:

R.H. (%)		Mean period (sec.) between take-offs per aphid
Batch A	50	38
	90	44
Batch B	50	61
	75	64

The effects of changes in relative humidity were further investigated in an experiment in which counts of flights were made 2, 5, 10 and 20 min. after changing the humidities (Fig. 15). The retarding effect of a change to a higher humidity and

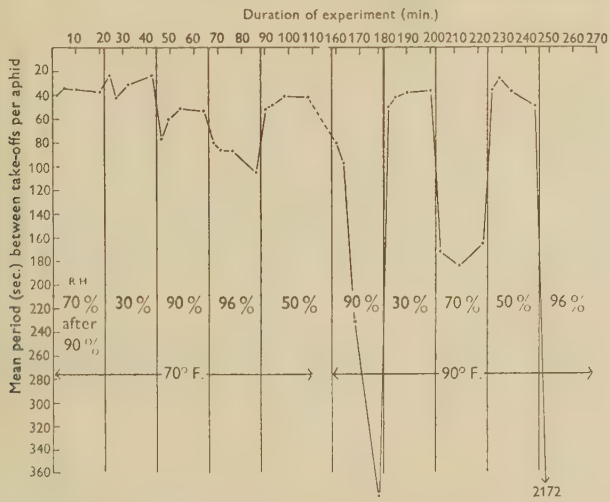

Fig. 15. The effect of changes of relative humidity on the activity of *M. persicae* at two temperatures.

vice versa was again clearly shown and was more marked at 90° F. than at 70° F. Solomon (1945), using papers impregnated with cobalt salts as indicators of humidity, found that the time required for the papers to reach equilibrium was much greater at very high humidities than at lower. The activity-retarding effect of changes by aphids to high from lower humidities might be analogous to the phenomenon described by Solomon, viz. a colloidal system taking longer to reach equilibrium with its surroundings at high humidities than at low.

Two experiments showed that the effects of changes in relative humidity did not persist. In one experiment three batches of *M. persicae* of the same age were confined in the chamber for 2 hr. at 70% R.H. and then tested. One batch was left at 70%, one was changed to 95% and one to 50% R.H. They were then tested after 2, 3 and 4 hr. (Fig. 16*a*). In a similar experiment two batches of *M. persicae* and

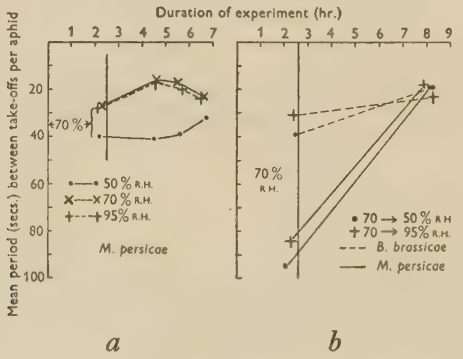

Fig. 16. The activity of aphids at different relative humidities.

B. brassicae were confined for 2 hr. at 70% R.H., then one of each was changed to 50%, the other to 95% R.H., and tested after 5½ hr. (Fig. 16*b*). In both experiments relative humidity had no effect on flight over a long period.

In another experiment four batches of eighteen *M. persicae* of the same age were tested at different humidities over a period of 10 hr. (Fig. 17*a*, *b*, *c*, *d*). Counts of flights were made 5–10 min. and 30–60 min. after each change. The effects of humidity were small (Table 9). Again, changing to a higher humidity retarded

TABLE 9. *Mean periods (sec.) between take-offs per aphid at the different relative humidities 30–60 min. after the changes were made*

R.H. (%)	Batch 1	2	3	4	Mean
50	71	56	45	75	62
70	81	26	69	47	56
90	80	27	70	62	60
100	82	34	93	47	64

activity temporarily, changing to a lower increased it. The counts 5–10 min. and 30–60 min. after the changes were expressed as multiples of the readings before the changes. The mean figures were:

	5–10 min.	30–60 min.
Changes to a higher humidity	1·20	0·92
Changes to a lower humidity	0·79	0·75

Similar figures from the previous experiments were:

Changes to a higher humidity = 1·25 × previous time
Changes to a lower humidity = 0·91 × previous time

Thus a change to a higher or lower humidity retarded or increased activity on the average by about 20% respectively. This effect was soon lost, as is shown by the figures for readings after ½ hr.

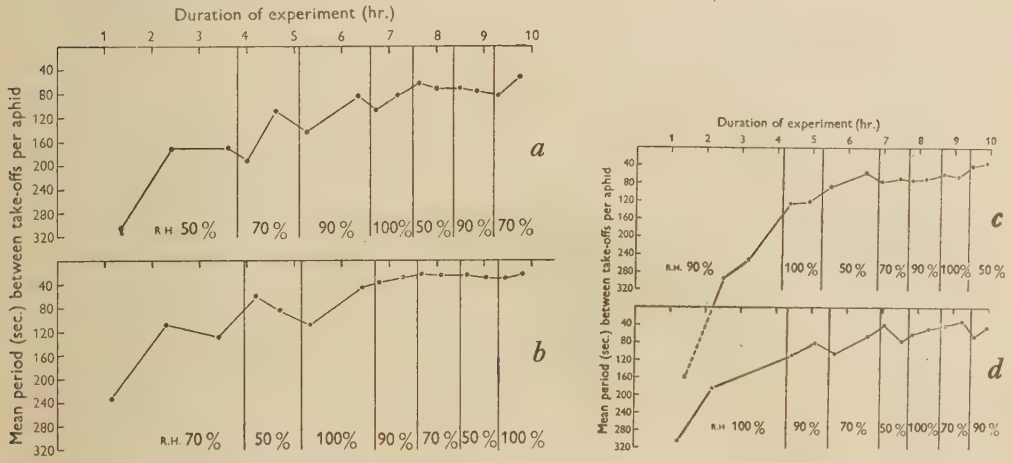
Duration of experiment (hr.)
Mean period (sec.) between take-offs per aphid
a
R.H. 50% 70% 90% 100% 50% 90% 70%
b
R.H. 70% 50% 100% 90% 70% 50% 100%
c
R.H. 90% 100% 50% 70% 90% 100% 50%
d
R.H. 100% 90% 70% 50% 100% 70% 90%

Fig. 17. The activity of *M. persicae* at different relative humidities.

These results explain why Davies (1935) thought that flight in *M. persicae* is inhibited at relative humidities over 70%. Davies used different batches of aphids of unknown age for each humidity, which, as has been shown above, may give very misleading results; the aphids were left at the temperature of the experimental room for ½ hr. before being introduced into the experimental chambers and were then left for 7 min. before records of flight were taken over a period of twelve consecutive minutes. Thus all the readings were made within 20 min. of the aphids being introduced into the chambers. Davies does not state the humidity at which the aphids had been kept prior to introduction into the chambers, but presuming that it was in the middle range, introduction into a low humidity chamber would result in much greater activity than introduction into a high humidity chamber and would account for the results obtained.

TEMPERATURE

The lower threshold of flight activity of *M. persicae* was found to be about 55° F. (12·8° C.), which confirms Davies's results. A batch of aphids which had been 2 hr. at 65° F. (18·3° C.) and 60% R.H. were transferred to the chamber at 55° F. and 30% R.H. and observed for 15 min. There was little movement, one or two aphids crawling slowly. When the temperature was raised 1° to 56° F. most of the aphids began crawling and occasional flights were made. Another batch of *M. persicae* was left overnight in the cooled chamber (55° F.) at 70% R.H. When the light was switched on next morning they made flights at this temperature. The chamber was then cooled to 51° F. (10·0° C.), at which no movement took place. Under conditions of slowly increasing temperature slow crawling began at 52° F. (11·1° C.) and fast crawling by some took place at 54° F. (12·2° C.). At this tempera-

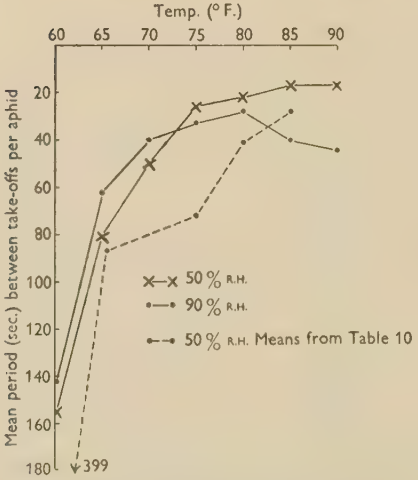
Temp. (° F.)
60 65 70 75 80 85 90
Mean period (sec.) between take-offs per aphid
20 40 60 80 100 120 140 160 180
399
50 % R.H.
90 % R.H.
50 % R.H. Means from Table 10

Fig. 18. The activity of *M. persicae* under conditions of increasing temperature.

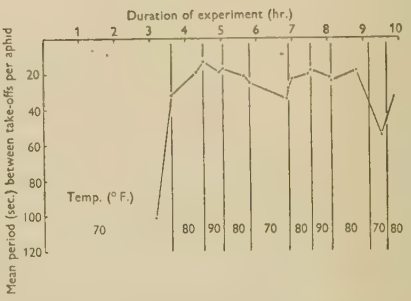
Duration of experiment (hr.)
1 2 3 4 5 6 7 8 9 10
Mean period (sec.) between take-offs per aphid
20 40 60 80 100 120
Temp. (° F.)
70
80 90 80 70 80 90 80 70 80

Fig. 19. The activity of *M. persicae* at different temperatures.

ture also a particularly large individual made the first flight and flew actively at 55° F. At 56° F. the others began to fly.

Two batches of eighteen *M. persicae* after 3 hr. at 65° F. (18·3° C.) and 60% R.H., were tested in the chamber at 50% R.H. and 60° and then 65° F. One batch was then transferred to 90% R.H. and both were tested at 5° F. intervals as the temperature was raised. An average of 10 min. was allowed for the temperature to rise 5° F. and the aphids were left at the new temperature for 5 min. before observation. Activity increased rapidly to 80° F., after which there was little increase at 50% R.H. and a decrease at 90% R.H. (Fig. 18).

In another experiment two batches of *M. persicae* and two of *B. brassicae*, all –2 days old, were tested while the temperature was raised from 62° F. (16·7° C.)

to 85° F. (29·4° C.) and lowered back to 66° F. (18·9° C.) over a period of 9 hr. The results after at least 35 min. at each temperature are given in Table 10; the means for *M. persicae* are also shown in Fig. 18. *B. brassicae* were much more active than

TABLE 10. *Mean periods (sec.) between take-offs per aphid at different temperatures*

	62° F.	66° F.	75° F.	80° F.	85° F.
M. persicae (1)	357 →	81 →	104 →	55 →	31
		106 ←	51 ←	35 ↙	
M. persicae (2)	441	107	90	45	24
		55	41	28	
Mean	399	87	72	41	28
B. brassicae (1)	56	35	11	11	10
			37	24	
B. brassicae (2)	54	41	11	13	12
Mean	55	38	24	18	11

M. persicae at the lower temperatures, but whereas *M. persicae* which had been exposed to 85° F. (29·4° C.) were more active than before when the temperature was lowered again, *B. brassicae* were less active.

Four batches of *M. persicae* which had been in the chamber at 70° F. (21·1° C.) under conditions of changing humidity for 10 hr. were subjected to a rise in temperature to 90° F. (32·2° C.) and tested after 20 min. at this temperature (Table 11).

TABLE 11. *Mean periods (sec.) between take-offs per aphid at 70° F. and then at 90° F.*

	Batch				
M. persicae	1	2	3	4	Mean
R.H. (%)	50	70	90	100	—
At 70° F.	34	53	45	23	39
At 90° F.	26	33	45	12	29

Two other batches of each species were kept in the chamber at 70° F. (21·1° C.) for 3 hr. and then the temperature was successively raised and lowered, activity being assessed at 70° F., 80° F. (26·7° C.) and 90° F. (32·2° C.) on each occasion. One batch of *M. persicae* was observed while the changes were taking place and it was obvious that the response by the aphids to a change in temperature was very rapid, activity during the change being sometimes greater than when the new lower or higher temperature had been reached (Fig. 19). Again *B. brassicae* were much less active than they were previously after exposures to high temperatures (Fig. 20), and a high relative humidity (100%) at 90° F. (32·2° C.) caused a decrease in activity: thus whereas an increase from 80–90° F. at 50% R.H. resulted in an increase of activity from 20 to 13 sec. between take-offs per aphid, a similar increase in

temperature at 100% R.H. resulted in a decrease of 34 to 154 sec. Comparable figures for a batch of *M. periscae* were:

At 50% R.H. 26 to 26 sec.
At 100% R.H. 43 to 30 sec.

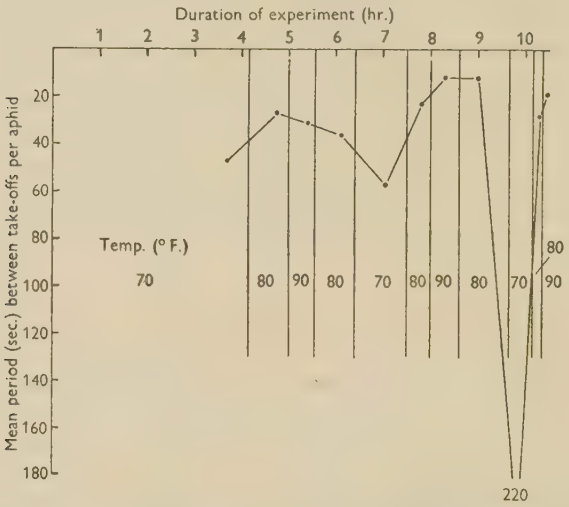
Duration of experiment (hr.)
1 2 3 4 5 6 7 8 9 10
Mean period (sec.) between take-offs per aphid
20 40 60 80 100 120 140 160 180
Temp. (° F.)
70 80 90 80 70 80 90 80 70 80 90
220

Fig. 20. The activity of *B. brassicae* at different temperatures.

PRESSURE

For experiments on the effects of pressure changes the aphids in the crystallizing dish were enclosed in a pneumatic trough within the experimental chamber. The trough was closed with a glass lid through which passed a tube connected to a mercury manometer and, via a two-way tap, to a filter pump for reducing the pressure and to a foot pump with a storage cylinder for increasing it. The lid was kept in position with a wooden frame. Experiments were performed with *M. persicae* after the aphids had been in the chamber between 4 and 5 hr. Changes in pressure consisted of $\pm \frac{1}{2}$ in. of mercury made rapidly; counts of flights were made immediately after each change and 5 min. afterwards. There was no evidence of very different activity at the different pressures (Table 12), but changes in pressure sometimes affected activity (Table 13).

The activity of the aphids was, on the average, the same before and after a reduction in pressure of $\frac{1}{2}$ in. of mercury, but the reduction caused a temporary increase in activity. On the other hand, an increase of pressure often caused an increase in activity; this was most marked with the increase in pressure from atmospheric to $+\frac{1}{2}$ in. of mercury. As changes in pressure sometimes affected activity it was to be expected that fluctuating pressure would have a similar effect. Aphids were observed at constant pressure and then while the pressure was fluctuating

TABLE 12. *Mean period (sec.) between take-offs per aphid after 5 min. at different pressures*

(No. of readings in brackets)

	Pressure (in. mercury)						
	$-1\frac{1}{2}$	-1	$-\frac{1}{2}$	Atmos.	$+\frac{1}{2}$	$+1$	$+1\frac{1}{2}$
Batch 1 (seventeen aphids) (Atmos. pressure = 29·77 in.)	19 (2)	22 (4)	23 (4)	21 (7)	—	—	—
Batch 2 (nineteen aphids) (Atmos. pressure = 29·92 in.)	—	—	37 (3)	42 (11)	33 (6)	40 (2)	—
Batch 3 (twenty aphids) (Atmos. pressure = 29·01 in.)	56 (1)	54 (2)	60 (2)	67 (2)	66 (2)	67 (2)	58 (1)
Batch 4 (fourteen aphids) (Atmos. pressure = 29·40 in.)	—	—	41 (1)	50 (3)	48 (5)	48 (4)	44 (2)

TABLE 13. *Periods (sec.) between take-offs per aphid at different pressures: (a) before, (b) immediately after, and (c) 5 min. after a change*

(Summary of four experiments with four batches of *M. persicae*)

Pressure change (in. mercury). $-\frac{1}{2}$ in.

	$-1\frac{1}{2}$ in. from -1 in.			-1 in. from $-\frac{1}{2}$ in.			$-\frac{1}{2}$ in. from atmospheric			Atmospheric from $\frac{1}{2}$ in.			$+\frac{1}{2}$ in. from $+1$ in.			$+1$ in. from $+1\frac{1}{2}$ in.		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
	24	20	19	28	21	24	43	25	38	29	28	40	70	71	71	58	52	70
	24	19	19	20	18	24	38	35	37	29	36	38	48	44	51	44	42	51
	55	58	56	62	55	55	30	28	36	33	29	30	51	41	42	—	—	—
	—	—	—	—	—	—	18	16	28	51	45	50	—	—	—	—	—	—
	—	—	—	—	—	—	22	17	20	42	46	45	—	—	—	—	—	—
	—	—	—	—	—	—	66	51	62	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	45	38	41	—	—	—	—	—	—	—	—	—
Means	34	32	31	37	31	34	37	30	37	37	37	41	56	52	55	51	47	61

Pressure change (in. mercury) $+\frac{1}{2}$ in.

	-1 in. from $-1\frac{1}{2}$ in.			$-\frac{1}{2}$ in. from -1 in.			Atmospheric from $-\frac{1}{2}$ in.			$+\frac{1}{2}$ in. from atmospheric			$+1$ in. from $+\frac{1}{2}$ in.			$+1\frac{1}{2}$ in. from $+1$ in.		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
	19	23	20	20	21	21	38	34	40	56	35	29	61	76	63	63	56	58
	19	18	21	21	21	24	37	32	34	50	49	34	45	53	37	37	42	44
	56	61	52	52	53	58	36	25	30	49	46	38	—	—	—	—	—	—
	—	—	—	—	—	—	21	21	21	40	34	29	—	—	—	—	—	—
	—	—	—	—	—	—	24	22	20	40	39	29	—	—	—	—	—	—
	—	—	—	—	—	—	58	73	67	34	37	33	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	67	56	61	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	56	71	52	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	50	62	45	—	—	—	—	—	—
Means	31	34	31	31	32	34	36	35	35	49	48	39	53	65	50	50	49	51

Total means ... $-\frac{1}{2}$ in. (*a*) 40, (*b*) 36, (*c*) 41. $+\frac{1}{2}$ in. (*a*) 42, (*b*) 42, (*c*) 38.

between atmospheric and $\frac{1}{4}$ in. of mercury with a periodicity of about 12 sec. Two or four consecutive counts of five times the number of aphids in the batch (this was the method used in all pressure experiments) were made (Table 14). Activity under

TABLE 14. *The activity of three batches of* M. persicae *under constant and under fluctuating pressure*

Mean periods (sec.) between take-offs per aphid

	Constant pressure			Fluctuating pressure		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
	21	64	94	16	55	80
	23	62	96	16	69	82
	22	—	—	17	—	—
	20	—	—	18	—	—
Means	22	63	95	17	62	81
Return to constant pressure		92	130			

conditions of fluctuating pressure was greater than under constant pressure. Activity was much less than it was previously when a return was made to constant pressure following fluctuation.

DISCUSSION

The factors affecting aphid activity are very complex and the importance of each in relation to the others is difficult to assess. In the work here described no attempt was made to probe the underlying physiological causes of reactions to different environmental conditions. The importance of changes in environment was clearly demonstrated. With temperature, relative humidity and pressure the differences in activity shown between aphids in two stable environments were often less than those shown while the environment was changing. It is possible that the effects of changing pressure might be the result of indirect changes in temperature and humidity, although a reduction in pressure would tend to cool the insect thus causing a temporary decrease in activity. In fact, activity was temporarily increased by such a change, so that pressure itself appeared to exert some influence.

Aphid populations are usually low in humid regions or in wet seasons, but high humidity probably affects the population by depressing multiplication, rather than by inhibiting flight as was suggested by Davies (1935). Lathrop (1923) showed that with *Aphis pomi* Degeer under normal outdoor conditions there was a general correlation between atmospheric evaporation and rate of development; the greater the evaporation, the shorter was the development period. Temperature, however, showed a closer relation to the rate of development. de Long & Mathewson (1925) found that, with *Myzus houghtonensis* Troop, a high humidity combined with a low temperature was apparently the most important combination of factors for retarding development: optimum conditions were high temperature and low humidity.

Autumn egg-laying and development in spring are adversely affected in wet seasons, so that fewer and later spring migrants might be expected in the more humid areas. At present, however, the effects of water relations and temperature on the plant, the aphid pest and its parasites and predators are incompletely understood, if not largely conjectural.

The influence of certain environmental conditions on aphid movement and virus transmission may be inferred from the present series of experiments. It is probable that aphids do not take to flight in the dark; those caught in Williams's light trap (Broadbent, 1947) must have been stimulated to fly to the light as they descended from the air levels to which they had ascended the previous day or from nearby herbage upon which they were resting. After a period of inactivity during the night alatae might be stimulated to fly with the return of light and would do so when the temperature rose to 55–60° F. if other conditions were suitable. More active flight would result as the temperature rose. Aphids normally climb to the upper part of a plant to test atmospheric conditions before taking to flight. If a gradient in relative humidity existed between the soil and the air above the crop it might be expected that the aphid would be stimulated to greater activity as it climbed higher to the less humid region of the upper leaves. On the other hand, an aphid alighting would presumably plunge suddenly into the higher humidity near the leaf surface and its activity would be temporarily retarded. Thus while relative humidity apparently does not primarily affect aphid flight, the changes in humidity which an aphid experiences as it moves about might have an affect upon its activity. Clearly much more needs to be learned about the microclimates of crops in relation to the ecology of their pests.

It is likely that environmental factors, acting upon the aphids, greatly affect the dissemination of virus diseases. It has been shown (Broadbent & Gregory, 1948) that alatae are largely responsible for the spread of potato virus diseases and that the 'persistent' virus leaf roll and 'non-persistent' virus *Y* of potato frequently spread at different rates in different localities or in the same locality in different years, although the two diseases have a common principal vector, *M. persicae*. Short, frequent flights such as the aphids might make during calm, warm late evenings would appear to be ideal for the dissemination of 'non-persistent' viruses, where short feeding periods on infected and healthy plants might follow a period of starvation during daytime flight. Similarly on calm, cloudy days or in the early morning flights will tend to be of short duration and from plant to plant; later when the temperature has risen and the sun is high, aphids taking to flight will orientate themselves towards the sun and fly upwards, then often be caught in convection currents and swept from the field. In addition the plants might be more susceptible to infection in the early morning after a period of darkness (Bawden & Roberts, 1948).

The conditions suitable for the spread of 'persistent' viruses in the field are presumably very different from the above, for long infection feeding must be followed by a long feed on the healthy plant. The long infection feed could take

place during a night or a windy day unsuitable for aphid flight. Once infected, the aphid remains infective for a considerable time and would be in a position to transmit the virus when next reduced to immobility for a long period by darkness or adverse weather. Windy days in summer are frequently followed by calm nights; the calm early morning or late evening would enable the infective aphid to change its location from an infected to a healthy plant. Both very active young alatae and older, more sluggish alatae could spread 'persistent' viruses under these conditions, but presumably only the active young would normally transmit 'non-persistent' types.

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A THREE YEARS' SURVEY OF POTATO APHIDS IN NORTH-EAST YORKSHIRE

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(With 3 Text-figures)

This paper considers the suitability of north-east Yorkshire as a centre for the production of high-grade seed potatoes, taking the number of aphid vectors of virus diseases as the criterion. Counts of aphids were made during three consecutive seasons on selected fields on the higher land in this area. These counts are related to the various geographical, climatic and biological factors found in each field and conclusions are drawn as to the most suitable districts within the area and the type of field which is likely to prove most satisfactory for this crop.

Introduction

In 1945 the Ministry of Agriculture introduced a scheme under which potatoes, grown in Yorkshire on land over 400 ft. above sea-level, were eligible for the high-grade certificates ('A' and 'SS'). Previous to this, a few such crops had been grown in the Skipton area of the West Riding and in one dale in the north-east. The suitability of the East Yorkshire Wolds and of the Hambleton Hills was immediately considered and a number of inquiries were received from growers as to the most favourable localities for this type of crop. Although it was known that this district had held a high reputation for seed-potato production over a century ago, the planting of potatoes had for various reasons almost died out. When it was decided to investigate the area in July 1945, no potatoes were to be found on the high land.

The problem was of an advisory nature, namely to obtain information which would enable growers to select the most suitable districts within the area and if possible to reject any field in which there was likely to be an exceptionally high aphid count. It was decided to conduct a general survey of the aphids on potato crops being grown in what seemed to be the most suitable areas. Moreover, from his work on the certification of potato crops, the writer was able to supply some data on the spread of virus diseases within the crop and it was possible that the results of an aphid survey could be related to this information.

Of the existing surveys of potato aphids in this country, most have been confined to the wetter western half. These include the various studies by the late Maldwyn Davies (1939) and the survey by Jacob (1944) in the northern Province, of Staniland (1943) in the south-west and the more recently published work by Broadbent (1946) in Derbyshire. Of these, only the conditions met with by Jacob in Northumberland are at all comparable with those in the drier and colder area in Yorkshire.

AREA SURVEYED

Details of the area surveyed are shown in the accompanying map (Fig. 1). Positions of the aphid counts are marked by numbers in the group 1–26 in 1945, 31–43 in 1946 and 44–53 in 1947.

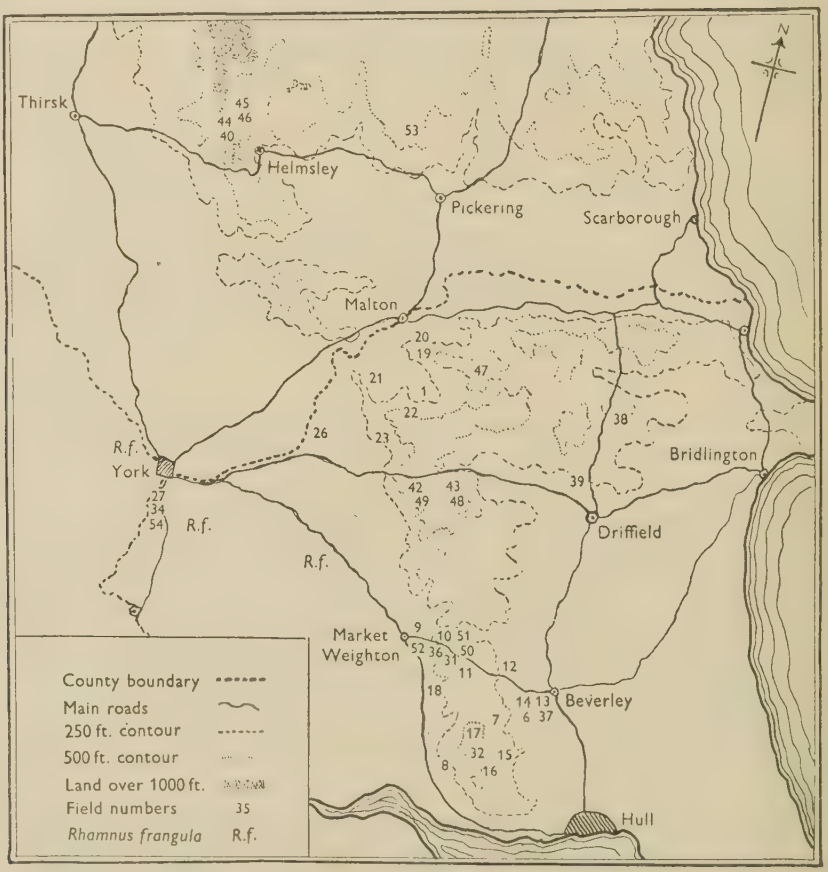

Fig. 1. Map of the area surveyed showing sites of the fields studied.

The East Yorkshire Wolds cover an area of about 480 square miles, of which rather more than 200 are over 400 ft. The highest points, just over 750 ft., are on the western edge which falls steeply to the Vale of York, the eastern slope being more gradual. These hills are typical chalk scarp land, the soil being mainly a flinty clay, often only thinly covering the underlying chalk. This soil is often very dry in the summer, thus producing conditions which encourage attacks of common scab (*Actinomyces* spp.).

The Hambleton Hills, farther to the north, are also scarp land, but less generally cultivated and rather higher than the Wolds, rising to over 1000 ft. at Sutton Bank. The soil differs markedly from that of the Wolds. The underlying rock is oolitic limestone and millstone grit, giving a soil which is often peaty, growing excellent potatoes, entirely free from scab.

As far as potato-growing is concerned, the whole area falls naturally into three main districts: (1) the southern Wolds, i.e. the area around and south of the Market Weighton-Beverley road, (2) the north Wolds, around and north of the York-Driffield road, and (3) the Hambleton Hills. Of these districts, only the southern Wolds is a regular potato-growing area and it lies generally lower than the other two.

Methods used in the survey

In July 1945 a study of the population trend of the aphids on a typical ware crop at Fulford (no. 27) was begun and when the numbers here reached a maximum, counts were made on as many as possible of the fields on the Wolds. The method used was that described by Davies (1939), i.e. of counting the number and species of aphids on 100 middle leaves, each from separate plants taken at random in traversing the field.

Examination of the figures thus obtained (Table 1) showed that the method was not entirely satisfactory. For example, it did not take into consideration the positions on the plant occupied by the various species. Further, it was noted that the standard index, i.e. the number of aphids per 100 leaves, made no allowances for the size of the potato plants, which varied from 22 to 120 leaves per plant, even at maximum growth. In the following 2 years therefore, the technique suggested by Thomas & Jacob (1943) was used. In this a top, middle and basal leaf from each of fifty plants are examined. On every fifth plant the flower head was taken instead of the top leaf.

In the potato field the plant is a basic unit of area and not the individual leaf, the number of which may vary, as pointed out above. In taking a fixed number of leaves—100 or 150—from a crop and counting the aphids thereon, a larger proportion per area is taken from a thin than from a dense crop, thus giving an exaggerated idea of the number of aphids moving from one plant to another. This is supported by examining the figures obtained in 1945 (Table 1), when the populations in the thin crops appeared to be generally higher than those on the more fully grown. It was decided, therefore, that a new index was required, giving the average number of aphids per plant throughout the field.

This figure could be calculated by simple proportion from the old index, arrived at from 150 leaves, if the average number of leaves per plant at the time of the aphid count was known. In the field this latter figure was not difficult to obtain. If the occasional bolters and dwarf plants are disregarded, and these should be negligible in a good seed crop, the size of the plants does not vary greatly throughout the crop and can be estimated with sufficient accuracy by taking the number of leaves per stem and stems per plant of about ten plants and working out the average.

The results of the survey are therefore stated throughout this paper as aphids per plant. This does not mean that these figures are not comparable with those obtained by other workers. It has been found that a fully grown maincrop potato plant in an average year contains roughly 100 leaves and therefore maximum population figures in the present survey should not differ widely from the standard index previously used. Moreover, it is understood that Mr L. Broadbent, working at Rothamsted over the same period, has reached very similar conclusions and is now also using the index of aphids per plant.

Subsequent to making the 1945 counts, it was realized that the peak population might not be reached at the same date in different fields. It was therefore decided that a number of visits should be made to each of about ten fields and the population

TABLE 1. *Results of survey, 9–12 August* 1945

Field no.	Locality	Altitude (ft.)	Aphids per 100 leaves	Crop density	Aphids per plant
1	Birdsall	200	12	Thin	7
6	Walkington	150	14	Thin	8
7	Lion's Den	375	78	Thin	44
8	S. Newbald	200	40	Dense	49
9	Weighton Hill	175	7	Medium	6
10	Weighton Wold	300	10	Medium	9
11	Arras	430	92	Thin	51
12	Gardham	300	24	Medium	22
13	Westwood (low)	60	24	Dense	30
14	Westwood (top)	125	4	Dense	5
15	Little Weighton	300	10	Dense	12
16	Double Dike	400	45	Thin	25
17	High Hunsley	530	6	Dense	7
18	N. Newbald	150	30	Dense	37
19	Langton Wold	325	6	Thin	3
20	Langton	225	16	Dense	20
21	Kenithorpe	210	4	Dense	5
22	Burythorpe	275	18	Medium	16
23	Leavening	250	2	Dense	3
26	Buttercrambe	50	15	Thin	8

trends observed, as had been done at Fulford in 1945. As far as other duties permitted, each field was visited at the middle and end of each month, starting at the end of June and ending early in September, visits thus being about 15 days apart. As all fields were visited at about the same date, it did not matter greatly if the exact peak was missed, since relative differences between field and field were of interest, rather than the absolute size of the population.

Where possible three fields were selected from each of the three main areas described above. This was not possible in 1946, since only one crop was then being grown on the Hambleton Hills. All fields were visited during the last week in June in both 1946 and 1947, but in no case were any aphids found at that date. Counts could seldom be continued after the first week in September, since the crops had usually either died down or had been burnt off by spraying.

Species found

Myzus persicae Sulz. appeared on every crop examined during the three seasons. *Macrosiphum solanifolii* Ashm. (=*gei* in Theobald) was common in 1946, but was much less plentiful in the other 2 years. Except at Fulford, *Aphis rhamni* Boyer (=*Doralis symphiti* Schr.) was only apparent in appreciable numbers in 1947, when it was found in most fields. *Myzus pseudosolani* Theob. (=*Aulacorthum solani* Kalt.) was found only in very small numbers at Fulford each season and at other areas hardly at all. *Myzus ornatus* Laing, which was found to be of some importance in the south-west by Staniland (1943) and recorded by Jacob (1944) in Northumberland, has not yet been found on potatoes in Yorkshire, although the writer has recorded it under glass.

Aphis fabae F. was found on potatoes in considerable numbers only in 1947. This species has not been included in the survey, since it is hardly a true potato aphid. Many specimens were found on weeds in the crop (mainly *Chenopodium* spp.) and the presence of this species in considerable numbers amongst the potatoes was of some ecological importance, as is noted below.

Results of survey

The examination of the potato crops at Fulford (nos. 34 and 54) was continued in 1946 and 1947, this district being typical of the ware-growing area in the Vale of York, and the results are plotted in Fig. 2. Fig. 3 shows the results of the survey on the other fields in 1946–7. These diagrams show that the form of the population curve is fairly constant for any given season, whatever the locality, and is typified by that at Fulford. The peak population and its position on the time scale, however, vary from field to field and the general shape of the curve is different from year to year and from species to species.

Myzus persicae

It is possible that the form of the development curve of *M. persicae* populations is due to three main factors: (1) arrival of migrants at the field in the spring, (2) rate of development of the aphids on the crop, (3) number of predators and parasites present. Broadbent (1946) has shown that in Derbyshire there was a further migration on to the potato crops during September. As stated above, in Yorkshire seed crops are not usually allowed to stand so late in the season and this migration cannot therefore be of importance here.

(1) *Spring migration.* This species is known to lay eggs on peaches and nectarines, but such plants are practically non-existent in East Yorkshire. Davies (1939), however, pointed out that *M. persicae* frequently survived the winter in the viviparous stage on Brassica crops, such as savoys and this was later confirmed by Jacob (1941). Savoys are not grown as farm crops on the Hambleton Hills, nor on the north Wolds, but can be found on the south Wolds in a few places. During the

winters of 1945–6 and 1946–7 observations were made on aphids found on savoys at Gardham (no. 12). Early in February 1946 the figure was as high as 4600/100 leaves, but severe weather at the end of that month reduced the numbers to 320 by early March; the crop was ploughed out early in April. Similar figures were obtained at Fulford under garden conditions. Few aphids can have survived the following winter in this manner, practically the whole of the savoy crop being destroyed by the very severe weather.

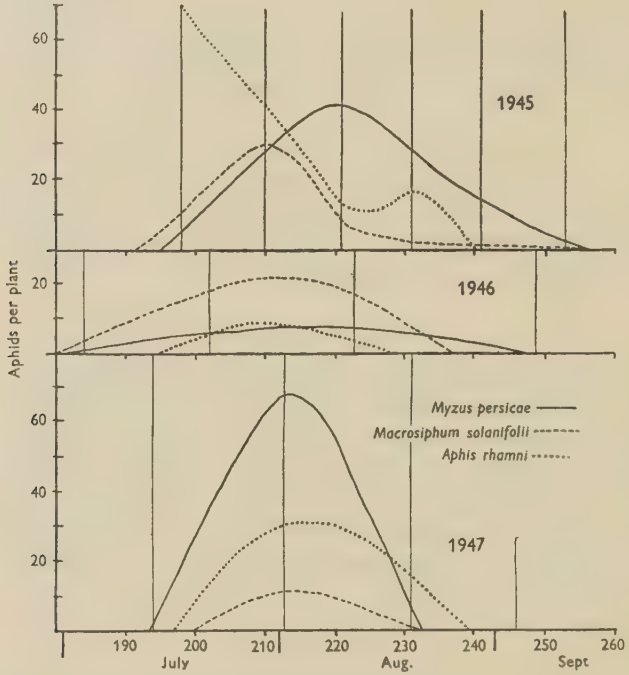

Fig. 2. Population curves of aphids at Fulford. Dates of counts are shown by vertical lines.

Broadbent & Hull (1947) have shown that *M. persicae* can overwinter in the viviparous form under certain conditions in mangold clamps, some of which may not be consumed until well into the summer months. Such clamps are, however, rarely used in the Yorkshire hills. Glasshouses, in which this species may also spend the winter, are restricted to the area around Hull. Probably the main sources are to be found in spring on Brassica crops in sheltered gardens and fields, particularly in the Vale of York.

The importance of the number which survive the winter should not, however, be overstressed, since it must be remembered that some 3 months may elapse between the consumption of the Brassica crop and the arrival of the migrating aphids on the

potatoes. During this period weather conditions suitable for building up a large population on weeds, etc., may or may not occur.

The suitability of weather conditions for aphid migration is of importance in considering the date and number of migrants arriving on the potatoes. Records at

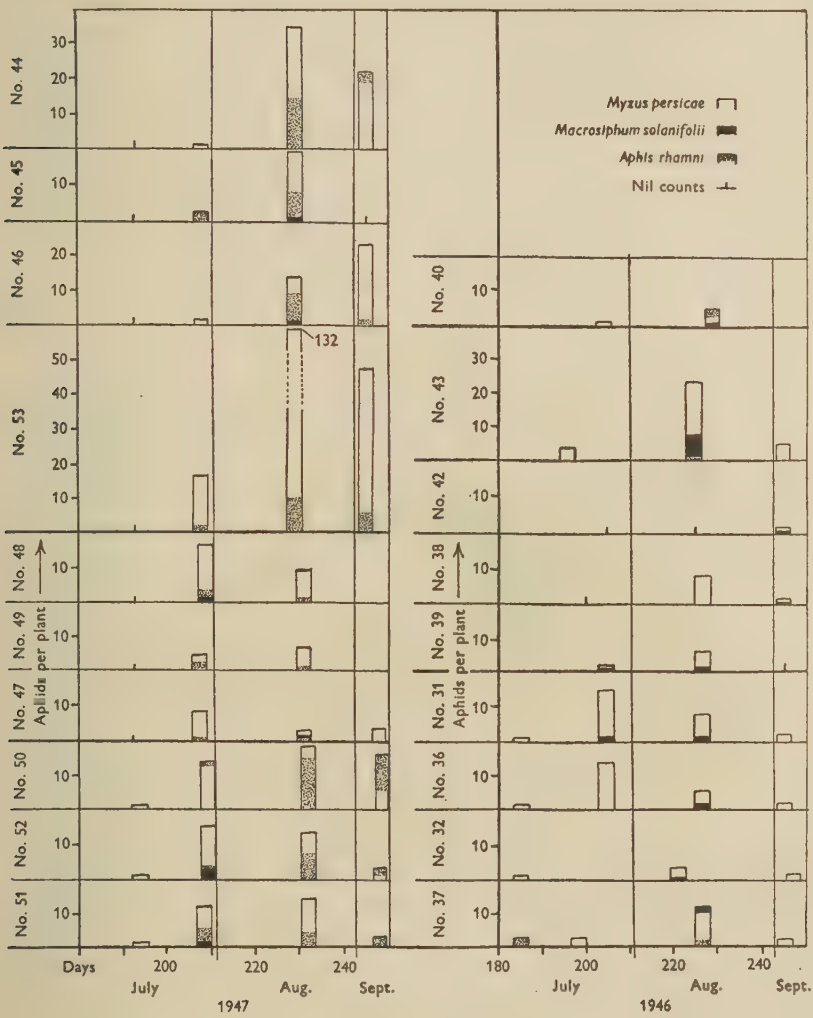

Fig. 3.

York Weather Station have been taken as sufficiently representative of those over the whole of the area surveyed. Comparing them with the conditions suggested by Thomas & Vevai (1940) as suitable for aphid flight, in 1946 only one day during

June (23) and four in July (9–12) were favourable. In 1947, on the other hand, 3 days were suitable at both the beginning and end of June, while after 12 July the weather was generally favourable, except for odd days.

The effect of these conditions on the aphid population figures may be seen in Figs. 2 and 3, and in Table 2, col. 5 of which gives the general proximity of the potatoes to a crop upon which *M. persicae* might have overwintered. With the fields on the south Wolds (nos. 31, 32, 36, 37 in 1946 and nos. 50, 51, 52 in 1947) *M. persicae* reached the crop by early July, whereas in other districts no aphids were recorded until the end of that month. As noted above, savoys are grown as a field crop in this southern area. In 1946 when migration weather was rare, the most isolated fields (e.g. no. 42) were not attacked by any aphids until the very end of the season. On the other hand, when after mid-July 1947 weather became favourable for flight, all fields had become infested by the end of that month.

The field at Huggate in 1946 (no. 43), although situated in an isolated district on the north Wolds, adjoined a kitchen garden containing Brassica crops. The results may be seen in Fig. 3.

(2) *Development rate.* The size of the peak population and the date at which it is reached, seem to depend mainly on the conditions within the crop itself. Unfortunately, little is known about these, because the microclimate within the crop is only distantly related to the weather conditions recorded at the local weather stations. A sheltered field is probably both warmer and wetter than one in a high and exposed position and the rate of aphid reproduction would therefore be higher in the former. Greater shelter will also be afforded at the base of the plant. Thus in 1946 *M. persicae*, taken over all the fields, showed an average of 23, 36 and 41 % of the population on the top, middle and basal leaves respectively, with a very similar figure for 1947. On the other hand, in the rather exposed field at Swaythorpe (no. 38) during the cold year of 1946, the percentages were 16, 16 and 60 respectively, while in 1947 at Cawthorne (no. 53), which was noted for its shelter, the figures were 32, 38 and 30.

Illustrations of the effects of a favourable microclimate can be seen in Fig. 3. Thus in 1946 only one field (Huggate, no. 43) showed considerable shelter from the very cold and wet conditions which set in after the middle of July and consequently, in comparison with other fields, a large population was built up there. In 1947 the effects of shelter were much less in evidence, owing to the generally more favourable weather conditions, and a rapid build-up was observed in most fields, particularly at Cawthorne (no. 53) which lay in a sheltered east and west hollow.

(3) *Parasites and predators.* Aphids are attacked by a host of enemies and these play a leading part in reducing the aphid population later in the season. Reliable methods of estimating this factor are lacking and recourse has to be made to general observations made in the field while the aphid population is being recorded.

During 1946, when the numbers of aphids on potatoes were very small, parasites and predators were very scarce and do not seem to have played a large part in reducing the aphid population, which gradually declined with the onset of colder

conditions. In 1947, as previously noted, many fields, especially weedy ones, contained in late July very large numbers of *Aphis fabae*. To these were attracted considerable numbers of parasites and predators which, when they had cleared the weeds of aphids, turned to the potatoes and rapidly reduced the *Myzus persicae*, etc. thereon.

Aphis fabae was noted on all the lower fields, particularly at Fulford (no. 54) and on the higher fields at Huggate (no. 48), Mowthorpe (no. 47) and Cold Kirby North (no. 45). The effects will immediately be seen from Figs. 2 and 3. As compared with the higher and more isolated fields, those at lower altitudes started a rapid increase of *M. persicae* during July. This development was, however, stopped short and reduced to relatively low figures by the parasites, etc. on the *A. fabae*. On the

TABLE 2. *The character of fields surveyed in* 1946 *and* 1947

Field no.	Locality	Altitude (ft.)	Aspect	Proximity to winter host
31	Gardham	350	Flat; open	Winter savoy crop in next field
32	Little Weighton	325	SW. slope; open	None near
36	Weighton Hill	275	SW. slope; rather sheltered	Town ½ mile W.
37	Westwood	100	S. slope; rather sheltered	Town ¼ mile E.
38	Swaythorpe	425	Slight N. slope; open	None near
39	Driffield	210	S. slope; very open	None near
40	Sutton Bank	1000	E. and W. slopes; very open	None very near
42	Callis Wold	750	Light N. slope; open	None near
43	Huggate	420	S. slope; rather sheltered	Kitchen garden adjoining
44	Sutton Bank	1000	E. and W. slope; very open	None very near
45	Cold Kirby (N.)	750	S.E. slope; open	None near
46	Cold Kirby (S.)	700	E. slope; rather sheltered	None near
47	Mowthorpe	600	S. slope; slightly sheltered	None near
48	Huggate	500	S.E. slope; very open	Village ½ mile S.
49	Callis Wold	750	Flat; open	None near
50	Gardham	350	Flat; open	Winter savoy crop in next field
51	Arras	450	Slight W. slope; open	Town 1 mile W.
52	Weighton Hill	300	W. slope; rather sheltered	Town ½ mile W.
53	Cawthorne	500	N. slope; very sheltered	None near

other hand, fields which were free from *A. fabae* and relatively isolated from introductions of both aphids and their enemies, started late, but towards the end of the season produced large populations, very large in fact where internal conditions were favourable.

This does not, however, explain the difference between the fields at Callis Wold (no. 49) and Sutton Bank (no. 44) which, although in different districts, were similar in the conditions so far considered, as seen from the close similarity in the form of the two population curves. The peak at Sutton Bank was, however, very much higher than that reached at Callis Wold. The Sutton Bank field stands on the very edge of the 1000 ft. scarp overhanging the Vale of York and it seems probable that after the middle of July, when conditions for aphid migration became exceptionally favourable,

the crop received a continuous supply of winged migrants carried up by the westerly airstream. This theory was supported by the rather larger proportion of alate aphids noted on this field and by recent observations with sticky traps. Callis Wold, on the other hand, lies about a mile back from the scarp edge and is very isolated.

Other species

Little is known of the detailed ecology of *Macrosiphum solanifolii* and it is not clear upon what plant this species spends the winter months. It is evident, however, from Figs. 2 and 3 that there is a general tendency for it to reach the higher crops at a later date, indicating that the winter host for this species should be looked for in areas at a low altitude.

Figures obtained in the present survey showed that in August 1945 this species was not present on any crop examined, except that at Fulford (no. 27). On the other hand, during the apparently much less favourable summer of 1946, it was found on all crops and at lower altitudes often reached figures higher than those for *M. persicae*. These figures, in fact, seem to indicate that there is a tendency for *M. solanifolii* populations, unlike those of *M. persicae*, to decline with a rise in altitude. *M. solanifolii* seems, therefore, to prefer damper, if not colder, conditions than do either of the other two species considered. This is also in some way confirmed by observations on the position of this insect on the potato plant. Thus in 1946, over all counts the average was 22, 23 and 55% on the top, middle and basal leaves respectively. In 1947, of the few specimens found, the figures were 43, 27 and 30%, which seem to confirm that this species is often found on the flower heads in fine weather.

In the ecology of *Aphis rhamni*, as described by Doncaster (1943), the overriding factor is the distribution of its winter hosts, *Rhamnus frangula* L. and *R. catharticus* L. Neither of these plants is at all common in north-east Yorkshire, the former growing only in rather boggy land at points marked (*R.f.*) on the map (Fig. 1). *R. catharticus* occurs as a hedgerow plant, but is found only very locally as isolated specimens.

Aphis rhamni has been recorded regularly only at Fulford (nos. 27, 34, 54) which, as will be seen from the map, lies between a number of *Rhamnus frangula* localities. In 1946 small scattered colonies were found on a few fields, but in 1947 this species was recorded on all crops and at times surpassed the figures for *Myzus persicae*. On the average far greater numbers of *Aphis rhamni* are found on the lower half of the plant, the figures being 19, 41 and 40% on the top, middle and basal leaves respectively; a habit which may be correlated with the increased numbers found in a fine summer.

The species is generally considered to be of less importance than the other two, although Kassanis (1942) has shown that it can be an efficient vector of virus *Y* in the laboratory. This may be partly due to the relative lack of mobility shown by the individual aphids of this species. The measure of this mobility is generally stated as the percentage of leaves occupied at each count. This index can be modified to show this habit even more clearly by stating the average number of leaves occupied by

100 aphids. Thus for *A. rhamni* this worked out at only 25·9 in 1946 and 38·8 in 1947, the difference probably being due to the much larger populations found in 1947. On the other hand, 100 *Myzus persicae* occupied as many as 68·3 leaves in 1946 and 58·2 in 1947, while in 1946 *M. solanifolii* was intermediate with 41·4 leaves. It is apparent from this, that compared with the other two species, *Aphis rhamni* shows a marked tendency to remain on the same leaf throughout its life.

Virus spread

The actual size of aphid population that will cause a virus spread sufficient to be of economic importance is not known with certainty. This is partly because there is little knowledge as yet of the conditions under which aphids spread virus in the field, or even of the factors which cause them to migrate within the crop. Davies (1939) suggested a figure of 20–100 aphids per 100 leaves, above which seed-potato production was not economically practical.

In 1945 the average figure for *Myzus persicae* was 17·9 aphids per plant and that season was noted for the very large spread of leaf roll that occurred in most crops. In 1946 the maximum *M. persicae* counts were down to an average of 9·0 per plant and inspections in 1947 showed that there had been on the average exceptionally little spread of virus the previous season. The figure for 1947 was 15·9, if the very exceptional field at Cawthorne (no. 53) is excluded, and this is correlated with a rather large spread of virus in most crops.

Figures for the spread of leaf roll are available from inspections under the certification scheme, for fields on the Hambletons and at Callis Wold (Table 3). They show that the low aphid counts in 1946 and at Callis Wold in 1947 are correlated with a virus spread of not more than 6–7 times, a figure which is quite controllable by roguing, if the initial stock is reasonably good. The Hambleton figures for 1947 of over twenty aphids per plant, resulted in a spread of leaf roll of as much as 35 times and a consequent rejection of the stock.

Table 3. *Spread of virus in* 1946 *and* 1947

		Leaf roll plants (%)			*M. persicae* per plant	
District	Variety	1946	1947	1948	1946	1947
Sutton Bank	A. Peak	0·009	0·055	—	1	—
,,	,,	0·010	0·065	0·050	1	34
,,	,,	0·020	0·030	1·0	1	34
Cold Kirby	K. Edward	—	0·010	3·5	—	21
,,	Redskin	—	0·055	0·5	—	19
Callis Wold	Majestic	0·50	1·0	—	$\frac{1}{6}$	—
,,	Red King	—	0·020	0·150	—	7

Conclusions

From the data recorded in this paper it is possible to draw some tentative conclusions on the suitability of the various localities in north-east Yorkshire. For example, it is apparent that seed production on the southern Wolds, with their crops of winter Brassicae, is not advisable, as in addition to the rather large aphid populations generally found there, these crops are usually infested early in July. This is of considerable importance, because the roguing of the crop for leaf roll cannot be completed much before the end of that month. It is essential that there should be very few aphids on the crop before all plants visibly affected with virus have been removed.

Except for occasional fields adjoining a kitchen garden or other source of early infection, potatoes on the Hambletons and the north Wolds are practically free from aphids until the end of July. It seems, however, that the north Wolds generally produce a lower maximum than the Hambletons, but as mentioned above, there is the danger of scab infection on the Wolds.

As to the variation between fields the matter is far more complex and less certain evidence is available. It seems clear that fields showing a marked degree of shelter, particularly from cold east winds, should be avoided, since they are generally liable to build up relatively large maximum aphid populations. If the field is also isolated from the winter host, this maximum, whatever its size, will be reached late in the season. On the other hand, Broadbent (1946) has pointed out that sheltered fields may miss the stream of migrants and receive fewer aphids from outside sources, than would an equally isolated but exposed field. Which is to be the controlling factor depends on the relative favourability of the weather during the spring migration and the period when the maximum is being built up.

It would thus seem that it is generally possible to account in retrospect for individual differences between one field and another, but they are connected intimately with the weather prevailing in the spring and summer months. In the light of existing meteorological knowledge it is not possible to make exact forecasts of the number of aphids, and therefore of the possible virus spread, to be expected in any particular field. It is usually possible, however, to indicate to a grower which of his fields is likely to prove most successful, or that his farm does not lie in a district suitable for seed-potato production.

The writer is particularly indebted to Mr H. W. Thompson for permission to continue these studies in 1947 after joining the N.A.A.S. Mr R. Wagstaffe, of the Yorkshire Museum, is also to be thanked for the meteorological records and other help, and Mr G. L. G. Jones for notes on the history of seed-potato production in Yorkshire.

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EXPERIMENTS WITH INSECTICIDAL SMOKES FOR INDOOR USE

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(With Plate 3)

Insecticidal smoke generators were tested in the laboratory and in practice. Each generator contained 110–120 g. of crude insecticide, either D.D.T. or benzene hexachloride. Over half the insecticide was evolved unchanged as a smoke of particles less than 1 μ in diameter, by a slow combustion mixture. In the laboratory tests, the generators were used at the rate of one to 1800–2500 cu.ft. (50–70 cu.m.). Benzene hexachloride had a greater initial insecticidal effect and a better residual action up to a week after treatment; thereafter (1 and 6 months) the D.D.T. was more effective. The residual effects of horizontal surfaces were good against bed-bugs, but the deposits on vertical surfaces were not highly efficient. Residual action against mosquitoes of vertical surfaces a week after treatment was fair, but effects of inverted surfaces were poor.

The deposits of active insecticide on horizontal surfaces were about 15–30 mg./sq.ft.; on vertical surfaces, 3–10 mg./sq.ft.; and on inverted surfaces, 2–6 mg./sq.ft.

A field trial was conducted between August and November in kitchens infested by flies and cockroaches. The generators were used at the rate of one per 64 cu.ft. (18 cu.m.). A substantial reduction of flies for 11 weeks was obtained by D.D.T., but for only 2 weeks with benzene hexachloride. Both treatments reduced heavy cockroach infestations to negligible proportions for 2–3 months. There was evidence of some repellent effect.

Thermal generators require careful precautions to prevent indirect tainting of food or beverages. They are very wasteful of insecticide especially since two or perhaps three treatments per season might be necessary. This would use twenty times as much insecticide as a spray treatment. On the other hand, labour and equipment costs are somewhat lower, and ease of operation very much greater, with smoke generators.

Introduction

During the war, small grenade-type insecticidal smoke generators were developed at the Chemical Defence Experimental Station, Porton (Ministry of Supply), for the rapid destruction of mosquitoes in malarious jungle. Subsequently, other uses of more general peace-time importance were investigated; in particular, room disinfestation by insecticidal smokes. For this purpose, the generators are operated

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indoors, in rooms with the doors and windows closed (but not specially sealed) for 1 or 2 hr. The advantages and disadvantages of the method may be summarized as follows:

(1) The procedure is simple and safe and requires no special equipment.

(2) The residual deposit produced is likely to be heaviest on the floor (as the smoke settles to the ground). This is a disadvantage, for the places frequented by insects (such as houseflies, cockroaches and bed-bugs) are the walls and ceiling; being vertical or inverted, these surfaces will collect relatively smaller deposits.

(3) The results of treatment are less disfiguring than a liquid spray application; but a fine bloom is left over polished furniture, floorboards and metal work. There is subsequently a more obvious smell of the insecticide used, and this may be distinctly disagreeable.

This paper describes experiments conducted to obtain further data regarding the feasibility of the method.

The generators used

The smoke treatments were all done with 'Generators, Insecticide No. 31, Mark I' (D.D.T.) or 'Mark II' (benzene hexachloride) the construction and performance of which are described by Bateman & Heath (1947). These contain 110 g. crude D.D.T. (70% *pp'* isomer) or 120 g. crude benzene hexachloride (10–12% *gamma* isomer), approximately 60–70% of which is emitted undecomposed as smoke particles of less than 1 μ in diameter. The smoke is generated by a slow combustion mixture which is ignited by a small priming charge. (More or less similar generators, ignited by a match flame, have since been produced by commercial firms.)

SECTION I: LABORATORY TESTS

(Conducted by J.R.B.)

A. Biological tests

1. *Methods*

The experiments were done in November 1945, at room temperature about 15° C. A room measuring $30 \times 7\frac{1}{2} \times 8$ ft. (1800 cu.ft.; 50 cu.m.) was used for the tests. For each test one grenade was set off in the centre of the room, all windows and the door being closed but with no further sealing precautions. The room was left closed for at least an hour, after which a substantial proportion of the smoke had settled or leaked away. Samples of the deposits were taken on horizontal and vertical glass slides and examined under the microscope (Pl. 3, figs. 1 and 2). With D.D.T. used as the insecticide, it was found that the particles deposited were liquid, apparently in a super-cooled condition, for mechanical stimulation (brushing with a paint-brush or even contact with a crawling insect) caused the rapid formation of needle-like D.D.T. crystals in place of the liquid. Where *gamma* benzene hexachloride (γ-B.H.C.) was used the deposit consisted of small dendritic growths of crystals.

(An interesting observation was the very extensive bushy growth of crystals of this insecticide along cobwebs. See Pl. 3, fig. 3.)

In both cases the quantities deposited on vertical or inverted surfaces were much less than those on normal horizontal surfaces (see p. 80).

2. *Tests of initial insecticidal action*

The penetrating powers of the smoke were roughly tested by exposing insects under one or two layers of straw matting (of the type used to protect wine-bottles). Bed-bugs were also exposed, unprotected, clinging to vertical strips of paper standing in Petri dishes. Mortality of mosquitoes was estimated 24 hr. later and bed-bugs 7 days later. The results were as follows:

TABLE 1. *Initial kills by insecticidal smokes*

Insect	No. used	Position	Percentage kills obtained with D.D.T. smoke	Percentage kills obtained with γ-B.H.C. smoke
Culex molestus	20–30	Under 1 layer of straw	100	—
Culex molestus	20–30	Under 2 layers of straw	100	100
Cimex lectularius	10–20	Under 1 layer of straw	30	90
Cimex lectularius	10–20	Under 2 layers of straw	8	90
Cimex lectularius	10–20	Exposed on vertical paper	90	100

3. *Tests of residual insecticidal action*

During the smoking trials pieces of Essex board (1 ft. square) and smooth blocks of Keen's Cement were placed in various positions in the treated room. Insects were subsequently exposed to these sample materials for various periods at different intervals afterwards. Throughout the rest of this paper the various surfaces will be described as 'horizontal' (where the *upper* surface was investigated), 'vertical', or 'inverted' (where an *under* surface was tested).

The results were as follows:

(*a*) *Bed-bugs*

TABLE 2. *Residual insecticidal effects of smokes on the bed-bug* (Cimex lectularius)

Type of surface	Insecticide	Exposure (hr.)	Percentage kill of bugs when exposed after 1 day	1 week	1 month	6 months
Horizontal	D.D.T.	6	55	86	—	77
,,	,,	17	100	100	100	75
,,	,,	24	100	100	95	94
,,	γ-B.H.C.	6	87	72	—	—
,,	,,	17	98	89	26	—
,,	,,	24	100	97	5	—
Vertical	D.D.T.	6	4	24	—	5
,,	,,	17	33	52	11	28
,,	,,	24	36	47	22	6
,,	γ-B.H.C.	6	27	8	—	—
,,	,,	17	45	12	12	—
,,	,,	24	84	22	11	—

(*b*) *Mosquitoes*

Since mosquitoes rest mainly on vertical or inverted surfaces, horizontal surfaces were not included in the tests. The exposures were made 1 week after treatment and the results are recorded in Table 3.

TABLE 3. *Residual insecticidal effects of smokes on mosquitoes*

			Aëdes aegypti				*Culex molestus*			
		Expo-	Survived		Died			Survived	Died		
Type of surface	Insecticide	sure (hr.)	♂	♀	♂	♀	Deaths (%)	♂ ♀	♂	♀	Deaths (%)
Vertical	D.D.T.	3	9	27	21	26	57	—	—		—
,,	,,	6	0	0	8	53	100	0 5	2	8	67
,,	,,	17	0	0	30	48	100	0 0	3	65	100
,,	γ-B.H.C.	3	0	0	30	49	100	—	—		—
,,	,,	6	0	0	9	47	100	0 0	0	44	100
,,	,,	17	0	0	36	30	100	0 0	2	56	100
Inverted	D.D.T.	3	25	37	7	6	17	—	—		—
,,	,,	6	0	1	12	17	96	0 28	0	4	13
,,	,,	17	2	11	29	67	88	0 1	6	40	98
,,	γ-B.H.C.	3	2	3	75	79	94	—	—		—
,,	,,	6	0	0	22	53	100	0 2	3	40	95
,,	,,	17	0	0	38	45	100	0 0	1	35	100

It will be observed that the longest observed exposures from which there were subsequent survivors were:

	Aëdes aegypti		*Culex molestus*	
	D.D.T.	γ-B.H.C.	D.D.T.	γ-B.H.C.
Vertical	3 hr.	Under 3 hr.	6 hr.	Under 6 hr.
Inverted	17 hr.	3 hr.	17 hr.	6 hr.

B. CHEMICAL ESTIMATIONS OF DEPOSITS

After these biological tests had been completed it was decided that some chemical determinations of the quantities of insecticide deposited by these smoke generators would be desirable.

1. *Method*

As it was impossible to use the same room, another was selected which measured $12\frac{1}{2} \times 18\frac{1}{2} \times 11$ ft. (2500 cu.ft.; 70 cu.m.). The deposits were collected on pieces of thin 'carbon copy' paper (measuring one-third and two-thirds of a square foot) pinned to squares of Essex board. These papers were disposed in horizontal, vertical and inverted positions at various heights from the floor, before discharge of smokes from the generators (one grenade per test). The papers were collected $1\frac{1}{2}$ hr. after release of the smoke, cut into small pieces and the insecticide removed by washing with hot benzene in Soxhlet extractors. After evaporation of most of the benzene and dehydrochlorination by caustic potash, the insecticide present was estimated by Volhard chloride determination.

2. *Results*

The following table gives the estimated deposits in mg./sq.ft.

TABLE 4. *Chemical estimations of deposits from insecticidal smokes*

(The figures indicate mg. insecticide per sq.ft.)

Height from floor	D.D.T. smoke (2 tests): position of paper			γ-B.H.C. smoke (1 test): position of paper		
	Horizontal	Vertical	Inverted	Horizontal	Vertical	Inverted
9–11 ft.	20·4, 25·7	3·8, 5·9	3·9, 5·5	30·9	10·1	6·0
6– 7 ft.	13·0, 14·8	3·9, 8·5	3·9, 4·4	17·3	3·0	2·1
3– 4 ft.	14·0, 18·5	3·2	3·2	19·5	—	—

SECTION II: A FIELD TRIAL

(Conducted by J.S.K.)

1. *Description of premises*

The trial was conducted in the mess kitchens of one of the Services at Old Sarum, Wiltshire. One kitchen, A, was left untreated as a control; another, B, was treated with benzene hexachloride and the third, C, with D.D.T. smoke. Kitchen B consisted of two rooms communicating through a wide permanent opening; a main kitchen $17\frac{1}{2} \times 27 \times 12$ ft. high and a smaller room $14 \times 18\frac{1}{2} \times 10$ ft. high; total volume 8900 cu.ft. (252 cu.m.). Kitchen C consisted of three rooms, two of which communicated through a wide permanent opening, the third communicating with one of the others through an ordinary door. The dimensions of the three rooms were: main kitchen, $30 \times 18 \times 10$ ft. high; preparation room, $27 \times 11 \times 10$ ft. high; washing-up room, $22 \times 10 \times 10$ ft. high; total volume of these three, 10,800 cu.ft. (306 cu.m.).

All the walls were of white glazed brick, but furniture provided a large area of other surfaces such as painted and unpainted wood and metal-work. The floor and working surfaces were washed down at least once daily and the walls of kitchen B were also washed daily, in so far as they could be reached from the floor. In both kitchens, the entire wall surface was washed down every few weeks. These activities were continued after smoke treatment.

2. *Infestation*

Flies (*Musca domestica* L. and a few *Fannia canicularis* L.) were common in all other kitchens before treatment. In addition, both the treated kitchens were heavily infested with cockroaches: *Blattella germanica* L. in kitchen B and *Blatta orientalis* L. in kitchen C. There were no cockroaches in kitchen A. An indication of the pre-treatment cockroach infestation densities may be gained from the fact that, in kitchen B, hundreds of *B. germanica* could be seen at a glance when the lights were suddenly turned on at night, thirty to forty insects being found running over one roller towel.

In kitchen C, the sudden turning on of the light at night revealed about thirty or forty *B. orientalis* at a glance.

3. *Methods employed*

(*a*) *Estimation of fly density*

In order to obtain some quantitative estimate of the effects of the treatment on fly density, a number of tanglefoot fly-papers were hung up in the various kitchens in certain fixed positions. Four papers were hung in kitchen A, seven in B and eight in C. The papers were set up 10 days before the treatment and then removed for counting; fresh papers were hung up immediately after treatment and renewed every 10–13 days for 11 weeks following the trial. Difficulties arose through unauthorized removal of some papers by Service personnel. Eventually it was decided to base the results solely upon papers which had caught over 100 flies in the 10 day pre-treatment period and which had been removed only once or twice during the subsequent assessment. The numbers of these more reliable papers were one in kitchen A, four in B and three in C.

(*b*) *Smoke treatment*

Treatment was carried out on the evening of 24 August 1945, after work in the kitchens had ceased for the night. Before the release of the smoke, exterior doors and windows were closed, but doors between treated rooms were left open. All food was removed together with loose kitchen utensils, but in kitchen C a large stack of crockery was left covered with a cloth. The generators were distributed as evenly as possible, fourteen Mark II (γ-B.H.C.) in kitchen B (that is, one to each 63 cu.ft. or 18 cu.m.) and sixteen Mark I (D.D.T.) in kitchen C (that is, one to each 67 cu.ft. or 19 cu.m.).

When all was ready the generators were rapidly set off by two operators and the outer doors closed for 1 hr. There was considerable visible leakage of smoke from the kitchens through the cracks in closed windows and doors. In addition, a good deal of smoke escaped up a service hatch in kitchen B, sufficient to cause discomfort to people in the floor above. At the end of the hour's exposure, smoke had largely cleared in kitchen B but was still thick in C; this was probably not due merely to greater leakage since the γ-B.H.C. smoke was observed to clear more quickly than D.D.T. even in laboratory tests with no leakage.

4. *Results*

(*a*) *Appearance of deposits*

The D.D.T. smoke deposit in kitchen C was visible as a 'bloom' on polished surfaces and a lightening of dark surfaces, but it was not disfiguring. It was greasy to touch and appeared to be liquid in most places. There was evidence of crystallization on spiders' webs, for they had a frosted appearance and the 'frosting' dropped off on jarring the web. The benzene hexachloride deposit in kitchen B was

more noticeable, particularly so as massive frosting of spiders' webs, and appeared to be in crystal form on all surfaces. All exposed iron or steel surfaces in kitchen C became markedly oxidized during the night following smoke treatment. This was not noticeable in kitchen B.

(*b*) *Human reactions*

When kitchen B was entered, at the end of the hour of B.H.C. smoke treatment, there was some irritation of nose and throat, but the atmosphere was tolerable. In kitchen C, however, the D.D.T. smoke was intolerable, the eyes being particularly affected as well as the nose and throat. In the first few days after treatment, there were numerous complaints by people working in or consuming food prepared in the treated kitchens. The tainting of food and drink by both insecticides was undoubtedly the most important matter; the residual odour would scarcely have caused objection had it not been associated with the unappetizing flavour imparted to food and drink. Most of this tainting was finally traced to particular sources. Water left overnight in large boilers in kitchen B became contaminated with B.H.C., though covered with a loose metal cover, and this tainted beverages made from it. Otherwise complaints arose mainly from a hot cupboard with a greasy inside which was not cleaned out after the treatment. Apparently the grease retained B.H.C. and gave it off slowly so that food stored in the cupboard became tainted long after the odour had disappeared from the rooms. Some trouble was also experienced from the crockery covered by a cloth during the D.D.T. smoke treatment in kitchen C. It was found that soapy hot water was necessary to remove the taint.

It will be noted that much of the contamination of food and drink could have been avoided by suitable precautions.

(*c*) *Destruction of insects*

(i) *Houseflies*. The numbers of flies caught in the first 10 days (before treatment) were as follows:

		Mean/paper/day
Kitchen A (1 paper)	146	14·6
Kitchen B (4 papers)	135, 271, 191, 182	17·5
Kitchen C (3 papers)	252, 135, 220	20·2

Thereafter, the numbers are shown in Table 5 and simplified by conversion to percentages. The 'expected' figures were calculated to take into consideration the seasonal decline in numbers of flies manifest in the untreated room. These data were calculated as follows: the number of flies in the untreated control room (per paper per day) for any required observation period was multiplied by the ratio of each treated room to the control room during the pre-treatment period. Thus in the period with mid-point at 9 days, the 'expected' number in kitchen B was $6{\cdot}2 \times \frac{17{\cdot}5}{14{\cdot}6} = 7{\cdot}4$ flies/paper/day. The number actually observed was 1·8, so that the percentage of expected was 24.

According to these results the benzene hexachloride treatment lost its effectiveness in 2 weeks after application whereas the D.D.T. smoke continued to have an effect up to the end of the trial (78 days). Dead or dying flies were not found in kitchen B after the first 2 weeks but they were observed, in small numbers, in kitchen C up to the penultimate trapping period. However, the numbers of dead flies found were always smaller than might have been expected from the reduction in numbers of those found alive.

If the general timing of changes in the fly population was reflected in the numbers trapped on the papers, the absolute fly density was not. Observers formed a firm opinion that there was a very much greater reduction in free flies than the reduction of flies trapped on the papers. It seems likely that the treatments had a repellent effect on the flies comparable with that observed on the cockroaches (see p. 84).

TABLE 5. *Mean number of flies trapped per paper per day after smoke treatment as percentage of pre-treatment numbers and as percentage of numbers 'expected'*

	A. Control		B. Benzene hexachloride			C. D.D.T.		
Mid-point of period	No. per paper per day	Percentage of pre-treatment	No. per paper per day	Percentage of pre-treatment	Percentage of 'expected'	No. per paper per day	Percentage of pre-treatment	Percentage of 'expected'
0·5	11·0	75	1·5	9	11	0·7	3	13
1·5	8·0	55	1·8	10	19	0·3	1	9
4·5	12·0	82	2·9	15	19	5·3	26	32
9·0	6·2	42	1·8	10	24	2·5	12	39
16·5	5·1	35	6·2	35	102	1·8	9	26
27·0	8·5	58	6·5	37	64	2·2	11	19
38·5	8·9	62	8·2	47	77	4·8	24	39
49·5	8·9	61	—	—	—	5·3	26	43
61·0	7·9	54	—	—	—	4·5	22	41
75·0	2·4	16	—	—	—	1·3	6	39

If the flies were activated by contact with the deposits and hence either alighted on the fly-papers or left the room sooner than they would normally have done, that would account for the shortage of free-flying or dead flies in the treated rooms (see Kennedy, 1947).

(ii) *Cockroaches.* Immediately after the treatment, dead and dying *Blattella germanica* were found in astonishing numbers in kitchen B. It was clear that the smoke had penetrated to the insect harbourages and driven them out into the open where they encountered the full concentration and fresh deposits of smoke. Very few of the moribund cockroaches were able to crawl and large numbers of egg capsules were being dropped.

During the next few days, further dead or dying specimens were observed and hardly any healthy adults were observed during the first 2 weeks. Thereafter an increasing number of young nymphs began to reappear, followed by the appearance of adults. At the end of a month the infestation in the treated rooms was probably

about a hundredth of the original numbers and the proportion of adults to young nymphs was about one to twenty. It seemed that some of the young nymphs were killed off before reaching maturity. At the end of 2 months the total infestation and proportion of adults had increased, but the infestation was still only about a twentieth of the original. After $3\frac{1}{2}$ months adults and nymphs were present in about equal proportions and the infestation was approaching pre-treatment level. The cockroach population of neighbouring, untreated rooms, that were infested before treatment, showed no marked reduction during the period, and this must have contributed to reinfestation of the treated rooms.

The course of events in kitchen C (D.D.T.) were generally similar, though the total numbers of *Blatta orientalis* were lower throughout. From the second week onwards until about $2\frac{1}{2}$ months after treatment cockroaches were extremely rare in both treated and untreated rooms. At $3\frac{1}{2}$ months, however, it was observed that a new brood of healthy insects, mainly nymphs, had become established in the treated rooms and it appeared that the infestation would reach pre-treatment density in a month or two unless further control measures were adopted.

In both kitchens B and C there was a sudden appearance of cockroaches in other adjoining parts of the building where none had been seen before. This was very marked during the first week after treatment, when some of the wandering insects showed signs of poisoning, though others were normal. This emigration from the treated rooms, for it could have been nothing else, ceased during the second week.

(iii) *Miscellaneous.* Immediately after the hour's smoke treatment a small number of silverfish (*Lepisma saccharina* L.), earwigs and unidentified moths were found in kitchens B and C. All the silverfish were dead. The moths were knocked down, but not all dead. None of the earwigs were dead, but they were more or less badly affected.

5. *Conclusions*

(*a*) This being one of the first trials of a new method, certain sources of contamination were overlooked and these gave rise to complaints of food and beverages. It is believed that these could have been obviated and that there would then have been no serious objection to the inevitable residual odour. In general the method was completely satisfactory in its convenience and simplicity.

(*b*) As regards efficiency, the reduction of flies by D.D.T. was about 60–80% for a month, and 60% for a further month and a half, according to the fly-papers. But direct observation indicated much more favourable results (nearly 100% reduction throughout the trial). At the very worst this suggests that three treatments per season would be required instead of the single treatment using the residual spray method.

(*c*) The cost of the generators used in this trial is not very important since a far less elaborate and much cheaper type can be made commercially available if a firm demand exists.

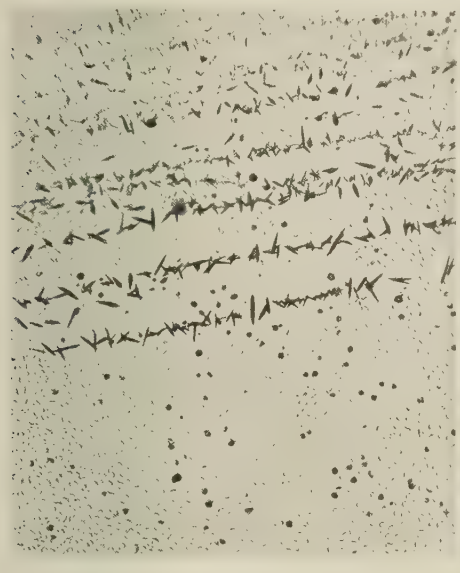

Fig. 1.

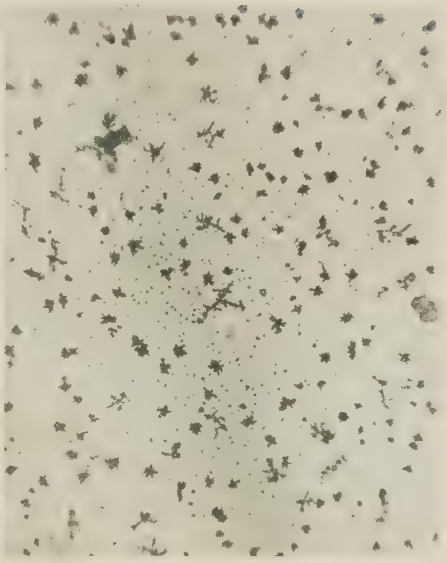

Fig. 2.

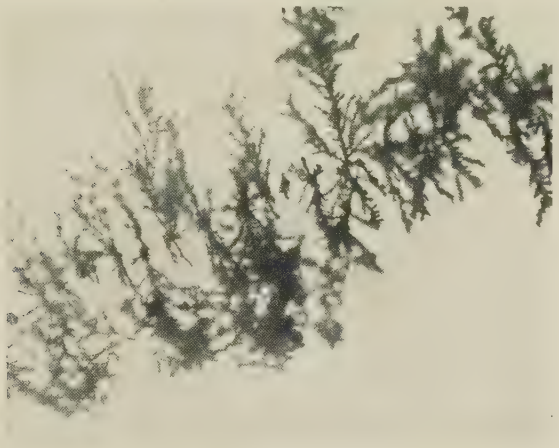

Fig. 3.

Nevertheless, it is clear that a much greater quantity of insecticide is required than is used in spray. In kitchen C a total of 1728 g. of D.D.T. were expended. With a spray treatment of all wall surfaces (at 100 mg./sq.ft.) only 228 g. would have been required; that is, about one-eighth as much as was used in a single smoke treatment.

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EXPLANATION OF PLATE 3

Fig. 1. Deposit of D.D.T. smoke on a horizontal glass slide. Over the top of the field crystallization has been induced by brushing with a small paint brush. Elsewhere there is some spontaneous crystallization. (Field approx. 1 × 1·5 mm.)

Fig. 2. Deposit of γ-B.H.C. smoke on a horizontal slide. Part of the deposit is in the form of supercooled liquid, but much has crystallized into dendritic growths. (Field approx. 1 × 1·5 mm.)

Fig. 3. Prolific branching crystals of γ-B.H.C. deposited on a spider's thread. (Field approx. 1 × 1·5 mm.)

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A TECHNIQUE FOR DETERMINING THE STOMACH POISON EFFECT OF INSECTICIDES USED AGAINST LEAF-EATING INSECTS

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(With Plate 4 and 11 Text-figures)

Methods devised for feeding individual insects with leaf areas bearing known deposits of insecticides are described, and the problems associated with incomplete consumption of the treated leaf portions are discussed. An account is given of the difficulties of applying the technique to small insects and to those with erratic feeding habits.

Experimental data are given to illustrate the effects on insect resistance of the following: (i) rearing conditions, (ii) larval age and body weight, (iii) acute and chronic poisoning, (iv) the diluting effect of the leaf tissue consumed with the poison dose.

The dosage mortality curve for stomach poisons was found to be typically sigmoid: analysis of results by the method of probits (Bliss, 1934) therefore proved satisfactory. Using lead arsenate against fifth-instar larvae of *Phlogophera meticulosa* the ratio of weight increase to increase in median lethal dose was found to be constant.

Introduction

Although the use of stomach poisons against insect pests is limited to those insects whose feeding habits enable them to consume the insecticide, the potentially greater efficiency by absorption through the gut wall in stomach poisoning compared with penetration through the external body wall in contact poisoning suggests that suitable stomach poisons provide the most effective means of control of mandibulate insects, particularly those attacking agricultural crops.

Until recently the use of stomach poisons was limited; arsenic and fluorine compounds were regulated in their use because of their danger to man and other animals, and organic insecticides such as nicotine and rotenone were often relatively ineffective. The discovery of new synthetic insecticides which act as powerful stomach as well as contact insecticides has greatly widened the field where stomach poisons may usefully be employed and has indicated the necessity for studying the purely stomach action of insecticides as distinct from their possible contact action. In the first place such a study can only be satisfactorily carried out by means of a laboratory technique where, under controlled conditions, the response of the insect to a known dose of insecticide can be accurately determined. The importance of laboratory techniques, in the determination of contact action of insecticides, is

emphasized by Potter (1941) and his considerations apply equally to the study of stomach insecticides.

A laboratory stomach poison technique involves several processes: (1) the accurate measurement of minute doses of insecticide for presentation to individual insects; (2) the consumption of known doses of insecticide by individual insects under conditions whereby contact action is as far as possible eliminated; and (3) the maintenance of treated insects under controlled conditions until all have either died or completely recovered.

Previous work on stomach poisons has been carried out mainly in the United States of America where several quantitative techniques have been developed. Campbell (1926*a*, *b*) used a microburette to measure droplets of insecticide solution which, when placed on the mouthparts of silkworm larvae, were rapidly imbibed. Such a technique is useful when accurate measurement of toxicity is required, but it is not intended, nor is it suitable, for a general study of stomach poisons, most of which are insoluble in water and would sediment inside the burette.

More widely applicable are methods whereby individual insects are induced to consume areas of food leaf upon which known weights of insecticide have been deposited. Methods used to deposit insecticides on foliage are described by Bezrukova (1937), Bulger (1932), Campbell & Filmar (1929), Stellwaag (1931) and Waters (1943) for dusts, and Trappmann & Tomaszewski (1937), McGovran & Meyer (1943) and Waters (1943) for sprays. Waters uses an upright celluloid cylinder 40 in. high × 8 in. diam. The insecticide is sprayed from above with several applications and allowed to settle on leaves on a spray platform. By careful manipulation deposits can be predetermined to within 5%, but no details are given regarding distribution of the deposit over the surface of the spray platform. An apparatus described by McGovran & Meyer consists of a large linoleum-lined cylinder 8 ft. high × 2 ft. diam. The insecticide is sprayed from below and allowed to fall back on leaves which are arranged on a spray platform around the atomizing nozzle. Distribution of deposit is somewhat uneven over the surface of the spray platform. No data are given on the error in reproducibility of deposits.

The question of the precision of spray towers is discussed by McCallan & Wilcoxon (1940) in connexion with the application of fungicides. Several spraying techniques are described and details given of their ability to reproduce a deposit. The most satisfactory apparatus is one which is similar in principle to that described in the present paper. It consists of a tower 5 ft. high and 1 ft. square within which the fungicide spray is allowed to settle. Deposits of various copper fungicides can be predetermined within 5·4%.

Methods of feeding treated leaf areas to the test insects are, several of them, modifications of the original leaf sandwich technique of Campbell & Filmar (1929), Stellwaag (1931) and Bulger (1937). Circles or squares of known area are cut from treated leaves and pairs made into sandwiches held together by paste and enclosing the sprayed or dusted leaf surface. A single leaf sandwich is fed to each insect.

Other methods (Hansberry & Richardson (1936), Ellisor & Floyd (1938), Ellisor & Blair (1940), Markos & Campbell (1943), Hansberry (1943) and Waters (1943)) utilize single strips or circles of leaf with the sprayed surface facing downwards or held in such a way that contamination of the body of the insect by the insecticide is as far as possible eliminated. Where necessary, though this applies mainly to dusts, the insecticide is held firmly to the leaf surface by means of a paste painted on the leaves before treatment. Mention should also be made of gross feeding techniques giving a considerable degree of precision (Potts, 1943).

In all these methods the actual dose of insecticide consumed is calculated from the area of leaf eaten. Generally the leaf area is not completely consumed and measurement of the amount eaten is necessary, for which three methods are used:

(1) Photographic enlargement and measurement with a planimeter (Campbell & Filmar, 1929).

(2) Photoelectric determination in which a photoelectric cell is used to measure the intensity of light which passes through holes in a partially eaten leaf area (Bulger, 1935).

(3) Direct measurement by placing the partially eaten leaf areas over millimetre graph paper (Hansberry & Richardson, 1936).

The present method consists of the feeding of treated leaf areas to individual test insects. Variation in insect feeding habits was such that no one method was found to be generally applicable. Furthermore, some insects with an erratic method of feeding could not be fed with quantitative doses by any method, while others were so small that the area of leaf consumed in a reasonable time was too minute to be estimated. Two feeding methods are described in the present paper—one suitable for small lepidopterous and coleopterous larvae and the other which is particularly successful for larger lepidopterous larvae. Other methods, both quantitative and qualitative are mentioned briefly.

The spraying apparatus

A laboratory spraying apparatus should fulfil the following requirements: (1) it should be capable of depositing any given weight of insecticide repeatedly on the spray platform; (2) the deposition over the spray platform should be even; (3) the spray platform should be large enough to carry a number of leaves.

Preliminary experiments were carried out with cardboard-lined spray towers to determine the most satisfactory type and size of apparatus. Finally, an apparatus was designed and constructed as shown in Text-fig. 1. It consists of a cylindrical spray tower resting on a table which carries the spray platform and atomizing apparatus.

The spray tower

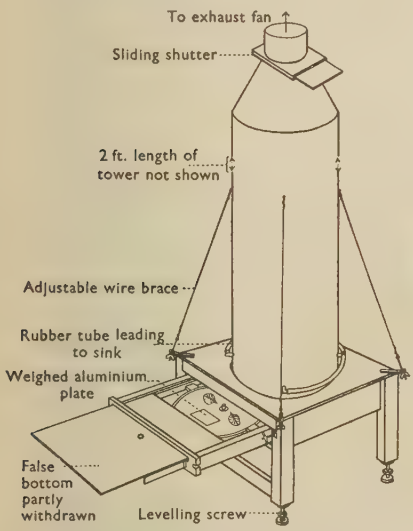
This consists of a cylinder 5 ft. 6 in. high, 1 ft. 3 in. diam. of thin sheet zinc (Text-fig. 2, *MT*) which is earthed to eliminate electric charge and lagged (Text-fig. 2, *L*) against outside temperature changes. At the top, a duct leads through an exhaust fan to the outside.

The tower rests on a rubber ring (Text-fig. 2, *RR*) on a flange surrounding a circular aperture in the table top. Four adjustable wire braces hold it in position and are used to correct any deviation from the vertical.

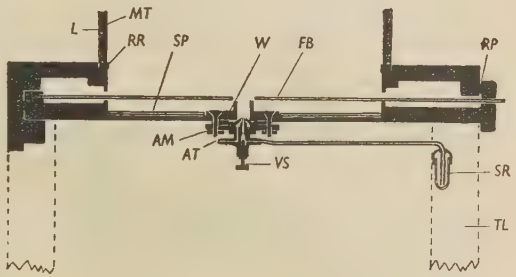
A rubber tube (see Text-fig. 1) leads to the sink from four $\frac{3}{10}$ in. diam. apertures around the base of the tower. These permit the escape of the air displaced during atomization.

The spray table

The table, which is supported by four 1 ft. legs (Text-fig. 2, *TL*) on levelling screws, is 2 ft. square with a 1 ft. 3 in. diam. central circular aperture over which rests the rim of the spray tower.



Text-fig. 1. Spraying apparatus for controlled deposition of insecticide sprays on leaf areas.



Text-fig. 2. Section through the spray table and the base of the spray tower. *AM*, mechanism for adjusting angle of spray stream; *AT*, compressed air lead to atomizer; *FB*, false bottom; *L*, lagging; *MT*, metal lining of tower; *RP*, rubber pad; *RR*, rubber ring; *SP*, spray platform; *SR*, spray reservoir; *TL*, outline of table leg; *VS*, valve; *W*, washer.

Set in the table is a drawer 1 ft. 10 in. long, 1 ft. 6 in. wide and $1\frac{2}{3}$ in. deep. The drawer, when closed, fits tightly beneath the top of the table with air-tight contacts at back and front maintained by pads of rubber tubing (Text-fig. 2, *RP*).

A false bottom (Text-fig. 2, *FB*) runs in grooves on the side walls of the drawer $\frac{9}{10}$ in. above the level of the true bottom. This consists of a sheet of 9 gauge glass 1 ft. 11 in. long and 1 ft. $6\frac{1}{2}$ in. wide with a 1 in. diam. hole in the centre which, when the false bottom is in position, lies directly over the atomizing nozzle. When required the false bottom can be withdrawn independently of the rest of the drawer.

The spray platform

The spray platform (Text-fig. 2, *SP*) is that part of the true bottom which, when the drawer is closed, lies directly beneath the spray tower. It is 1 ft. 3 in. in diameter

and is encircled by a strip of flat brass rod $\frac{4}{5}$ in. high. In the centre a brass tube $\frac{9}{10}$ in. internal diameter and $\frac{2}{5}$ in. high is fitted above the orifice of the atomizer. Its importance in preventing spray droplets from being thrown out on to the spray platform during spraying will be realized when the spraying technique is described. A 1 ft. 2 in. diam. glass plate covers the floor of the spray platform except in the centre where a 3 in. diam. hole allows part of the atomizing apparatus to project. The glass plate carries the leaves to be sprayed and can be rotated to facilitate the even deposition of spray.

The atomizing apparatus is fixed by three bolts to the underside of the drawer with the nozzle projecting upwards through the centre of the spray platform. The bolts work against a rubber washer (Text-fig. 2, *W*) and can be used to adjust the atomizing nozzle to throw a spray jet vertically up the centre of the tower (Text-fig. 2, *AM*).

A $\frac{1}{16}$ in. diam. liquid feed pipe fitted with a regulating valve (Text-fig. 2, *VS*) connects the liquid nozzle of the atomizer to a spray reservoir (Text-fig. 2, *SR*) beneath the front of the drawer.

Essential requirements for the nozzle are: (1) the rate of atomization should be about 1 c.c./sec. at an air pressure of 40–50 lb./sq.in. This figure is not necessarily the optimum, but slower rates of atomization are not suitable, particularly with suspensions of relatively large particles which sediment rapidly; (2) the spray jet should be narrow to reduce to a minimum the throw-out of spray on the side walls of the tower. A nozzle of the following dimensions is used: diam. of air orifice, 0·069 in.; diam. of liquid orifice, 0·026 in.; length of liquid nozzle projection beyond level of air orifice, 0·018 in.

Spraying technique

The leaf areas to be sprayed must be flat, to give equal distribution of deposit per unit area. Suitable leaf areas are placed on the glass plate of the spray platform together with a weighed aluminium foil sheet of known area (14 sq.in., see Text-fig. 1). This is weighed again after spraying and is used to check the exact weight of deposit obtained.

The spraying procedure is generally as follows: with the drawer closed and the false bottom in position, 5 c.c. of spray of known concentration are placed in the spray reservoir and immediately atomized at an air pressure of 45–50 lb./sq.in. The time taken to atomize is important and is always checked, any deviation being indicative of incorrect air pressure or nozzle blockage. The air line is turned off immediately after the 5 c.c. of insecticide has been completely atomized.

The false bottom is kept in position for 30 sec. following the beginning of atomization. During this time the larger droplets of spray settle out and the spray remaining in the tower becomes stabilized and evenly distributed. The false bottom is then withdrawn from above the spray platform thus exposing the leaves and aluminium plate to the settling spray. The spray can either be allowed to settle out completely

or the false bottom can be replaced before complete deposition, when the weight of deposit depends upon the length of time allowed between withdrawal and replacement. When the required deposit has been obtained and the false bottom replaced, excess spray in the tower is removed by means of the exhaust fan and it is only during this process that the shutter at the head of the tower is opened.

Generally the above procedure is repeated several times in order to obtain the total deposit required and, where possible, it is found most satisfactory to spray at least four times. After each spraying the glass plate on the spray platform is rotated through 90° and before continuing with the spraying procedure sufficient time is allowed for spray droplets deposited on the leaves to dry. When half the sprayings for the required deposit have been carried out, leaves on the periphery of the spray platform are interchanged with those near the centre. Although the method used increases the time taken to produce deposits it results in greater accuracy and evenness of deposition.

Performance of spraying apparatus

Certain data were obtained on the efficiency of the spraying apparatus, using the following preparations:

(1) A suspension of crystalline D.D.T. particles in water medium containing 0·1% w/v sulphonated lorol and 10% v/v acetone (McIntosh, 1947). Sprays of this insecticide settled rapidly after atomization.

(2) A suspension of pure lead arsenate in water which settled more slowly.

(3) A solution of pure D.D.T. in acetone which when atomized produced a very slow-setting D.D.T. aerosol.

In the first place the efficiency of the apparatus with the false bottom removed was determined by weighing the total deposit of an 0·2% w/v D.D.T. crystalline suspension of 60 × 15 μ crystals falling on glass plates 20 sq.in. in area.

The following procedure was used: a weighed glass plate was placed in a particular position on the spray platform and 5 c.c. of water were atomized into the tower with the false bottom closed. After 2 min. the spray had completely settled on the false bottom which was then removed. By this time it was considered that the atmosphere in the tower was saturated with water vapour and that further atomization would cause little additional vaporization. 5 c.c. of the D.D.T. suspension were then sprayed into the tower and the resulting mist allowed to fall on the glass plate. Deposition was complete after 2 min., the glass plate was quickly removed, placed in a watertight perspex holder and weighed. Twelve replications of this procedure were carried out, the weights of deposit obtained being shown in Table 1.

Such a spray deposit is considerably heavier than would be possible in practice. Much coalescence of droplets occurred which, as mentioned before, must be prevented if even deposition over small areas is required. However, the data show that a constant proportion of the spray sediments, i.e. about 32%, with this

insecticide. The proportion sedimenting would undoubtedly vary with the type of spray medium and the spraying technique used.

TABLE 1. *Replication of spray deposit of an 0·2% w/v D.D.T. crystalline suspension in water*

(Figures show weights of spray in mg. falling on a plate 20 sq.in. in area)

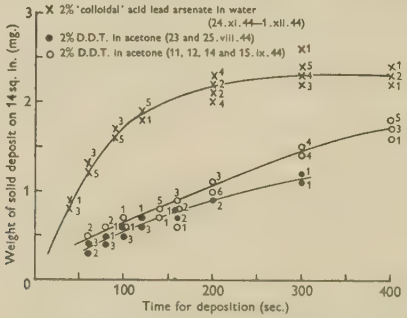
Volume sprayed, 5 c.c.; false bottom removed throughout spraying procedure; time for deposition, 2 min.; no. of sprayings for total deposit, 1

173·4	178·0
172·8	180·1
165·0	170·4
181·1	177·1
170·3	176·2
181·3	185·1

Mean and standard error = 175·88 ± 5·71.

Clearly in the normal spraying procedure weighing the total spray deposit is not possible because of errors caused by vaporization of the medium. Consequently the most satisfactory method is to determine the weight of deposit of the non-volatile constituents of the spray. Unfortunately, in many cases, the weights of solid required for toxicity tests are very small and in the following determinations a weighing error is therefore involved.

The following data on weights of deposit were obtained by using the standardized procedure previously described. First, variation in replication of deposit produced by single sprayings was determined. These were followed by detailed experiments on predetermination and distribution of deposit, using the full procedure, whereby four sprayings were carried out to obtain the total deposit required.



Text-fig. 3. Relationship between time for deposition and weight of single deposits of D.D.T. and lead arsenate. Figures denote the number of times that a particular deposit has been replicated. Volume sprayed, 5 c.c.; time to spray, 5 sec.; air pressure, 45–40 lb./sq.in.; false bottom in position, 30 sec.; number of sprayings for total deposit, 1.

Text-fig. 3 shows the weights of insecticide (pure D.D.T. and lead arsenate) deposited by single sprayings on a 14 sq.in. area of aluminium foil. The variation in

replication for a series of deposition times is shown, the figures against each point denoting the number of times that a certain weight of deposit has been repeated for a particular deposition time. In several cases a distinct variation from the expected weight of deposit is noticeable and it is the error caused by such individual variation which is practically eliminated by carrying out several sprayings for a required deposit.

In the experiments with D.D.T., data on weights of deposit were obtained on six separate days. Experiments on 23 and 25 August 1944 show weights of deposit consistently lower than in experiments on 11, 12, 14 and 15 September 1944. Temperature and humidity conditions in the spray room were determined. These did not vary widely (59–70° F. and 72–84% R.H.) and within these limits no correlation of weight of deposit with such conditions was noticeable.

Four experiments with lead arsenate carried out between 24 November 1944 and 1 December 1944 show no day-to-day variation in weights of deposit.

TABLE 2. *Distribution of deposit on spray platform obtained by spraying D.D.T. in acetone and lead arsenate powder in water*

(Figures show weights of solid insecticide in mg. on 2 in. square plates)

5% D.D.T. in acetone: volume sprayed, 5 c.c.; time to spray, 4·5 sec.; air pressure, 45–50 lb./sq.in.; false bottom in position, 30 sec.; time for deposition, 40 min.; no. of sprayings for total deposit, 4

7% lead arsenate in water: volume sprayed, 5 c.c.; time to spray, 5 sec.; air pressure, 45–50 lb./sq.in.; false bottom in position, 30 sec.; time for deposition, 5 min.; no. of sprayings for total deposit, 4

	D.D.T. in acetone				Lead arsenate in water		
No. of plate	18 Oct. 1945	20 Oct. 1945	24 Oct. 1945	29 Oct. 1945	31 Jan. 1945	31 Jan. 1945	1 Mar. 1945
1	6·50	6·40	6·65	6·60	2·4	2·4	2·5
2	6·65	6·35	6·55	6·60	3·04*	2·3	2·4
3	6·75	6·35	6·60	6·40	2·5	2·3	2·5
4	6·70	6·35	6·70	6·45	2·5	2·4	2·4
5	6·60	6·30	6·70	6·55	2·4	2·3	2·4
6	6·65	6·35	6·65	6·55	2·4	2·3	2·4
7	6·75	6·45	6·55	6·60	2·4	2·3	2·4
8	6·65	6·35	6·65	6·40	2·4	2·3	2·4
9	6·55	6·40	6·60	6·60	2·3	2·3	2·3
10	6·65	6·35	6·60	6·55	2·4	2·2	2·4
11	6·60	6·40	6·65	6·55	2·4	2·2	2·4
12	6·65	6·35	6·55	6·45	2·4	2·2	2·3
13	6·65	6·35	6·65	6·55	2·3	2·2	2·4
14	6·65	6·30	6·65	6·45	2·4	2·2	2·3
15	—	—	—	—	2·3	2·2	2·3
16	—	—	—	—	2·3	2·2	2·3

* A drop fell on this plate from rim of tower—not included in analysis.

Table 2 shows the results of more detailed experiments in which the total deposit was obtained by four separate sprayings using the standardized procedure previously described. Weighed aluminium foil plates, 2 in. square, were distributed on fixed positions on the spray platform, nos. 1–4 being 1 in. from the periphery, nos. 11–14

(for D.D.T.) and nos. 13–16 (for lead arsenate) being $2\frac{1}{2}$ in. from the centre and the rest $4\frac{1}{2}$ in. from the centre. 5% D.D.T. in acetone, and 7% powdered lead arsenate in water were used, each experiment being repeated four times with D.D.T. and three times with lead arsenate. In Table 2 differences in figures across the table are indicative of the error in replication, while figures down the table show evenness of distribution. Table 3 shows an analysis of the data obtained.

TABLE 3. *Analysis of results of experiments to determine distribution and replication of D.D.T. and lead arsenate deposits*

Insecticide		Mean weight of deposit (mg.)	S.E. in distribution	S.E. in replication
5% D.D.T. in acetone	18 Oct. 1945	6·643	±0·068	
	20 Oct. 1945	6·363	±0·040	
	24 Oct. 1945	6·625	±0·047	6·538±0·133
	29 Oct. 1945	6·521	±0·077	
7% Lead arsenate in water	31 Jan. 1945	2·39	±0·05	
		2·27	±0·07	2·35±0·07
	1 Mar. 1945	2·38	±0·06	

It is noticeable that weight of deposit of lead arsenate decreases from the periphery of the spray platform inwards. In practice, as previously described, this error is eliminated by interchanging leaves halfway through the spraying procedure.

With the present atomizer, when the false bottom was not used, larger droplets over small areas were noticeable and the deposit on the spray platform was unevenly distributed. For example, with a lead arsenate spray the deposit on the area surrounding the spray nozzle was sometimes 10% greater than over the greater part of the area of the spray platform, whereas on the periphery the deposit was consistently about 5% smaller.

General conclusions on the efficiency of the spraying apparatus are as follows:

(1) When the false bottom is used the standard error in distribution of deposit is about 5%. This error can be eliminated, as previously described, by interchanging leaves on the periphery and the centre of the spray platform halfway through the spraying procedure.

(2) For emulsions, solutions and suspensions with particle size less than about 50 μ the standard error in reproducibility is 5% or less.

When a suspension consisting of large particles is used, the rate of sedimentation may be such that it is complete within about 30 sec., and consequently it is necessary to reduce the time with the false bottom in position to a minimum. With a suspension of 150 μ D.D.T. needle-like crystals the procedure adopted was to spray 2 c.c. per application and remove the false bottom 5 sec. after the beginning of spraying. The deposits obtained in this way showed an error in replication of about 15% whilst a 10% error was obtained with a suspension of potassium fluoaluminate of particles up to 100 μ in diameter. In general it may be said that the more rapid the rate of sedimentation the greater is the error in predetermination.

(3) The apparatus is clearly not suitable for spraying of volative or unstable compounds, because of the delay occurring between deposition of the poison and consumption by the insects. It seems, however, probable that insecticides such as the pyrethrins may be sprayed in solution in a non-volatile oil without loss of toxic principles.

(4) Only a proportion of the insecticide sprayed into the tower is deposited on the leaf areas and fed to the test insects, the rest being either thrown out on the side walls of the tower, or falling on the false bottom. Consequently, with suspensions of varying-sized particles, there is a danger of segregation resulting in particle size of the deposit differing from that in the original spray. This has not been apparent so far, possibly because up to the present most of the preparations used have contained particles of relatively uniform size.

Technique for feeding treated foliage to individual insects

As previously mentioned, variations in size and feeding habits of leaf-eating insects are such that it is not possible to devise a standard technique applicable to all species. In any technique of this type two processes are involved:

(1) The removal of portions of treated leaf of known area.

(2) The enclosure of the small leaf areas with individual insects in such a way that contact of the insect with the treated surface is, as far as possible, prevented. An essential requirement is that the technique should be simple in that feeding appliances can be set up in a short time and a number of insects fed simultaneously.

Lepidopterous larvae have proved so far the most satisfactory test subjects because they consume completely the leaf areas presented to them. However, difficulties occur, particularly with small larvae such as those of *Plutella maculipennis* Curt., when speed of action of some insecticides is so great that the feeding of individual insects is arrested. The time taken to consume the required dose is the limiting factor, and it is necessary to select a strip of leaf of sufficiently small area for it to be completely consumed before the speed of action of the insecticide affects the feeding larvae.

Table 4. *Relation between weight of last-instar* P. maculipennis *larvae and extent of feeding on 2·2 sq.mm. leaf strips bearing 0·00052 mg. lead arsenate (13 July 1945)*

Average weight of larvae (mg.)	Average area of leaf consumed per larva (sq.mm.)	Average dose consumed per larva $\times 10^5$ (mg.)	Percentage kill
2·87	1·04	25	38·5
3·75	1·34	32	40·0
4·73	1·57	37	37·8
5·51	1·79	42	60·0

Table 4 shows results of an experiment with lead arsenate in which batches of forty last-instar larvae of varying body weight, starved for 2–4 hr. at 75° F. and 100% R.H., were fed individually on strips of cabbage foliage 2·2 sq.mm. in area

each bearing a deposit of 0·00052 mg. The area of leaf strip consumed by each larva before it ceased feeding was determined by measurement of the partially eaten leaf strip under a binocular dissecting microscope. During treatment the insects were kept in a constant temperature cabinet at 75° F.

It is clear from Table 4 that with lead arsenate the area consumed per larva is dependent upon the size of the insect, and also, if known doses are to be given, a very small area of treated foliage must be presented to the larva to ensure that the whole area presented is completely consumed. The size of leaf strip that can be used is limited by practical considerations—error in cutting strips of a known area, water loss, and distortion caused by handling, all of which become important as the leaf strip becomes smaller. As a result it is considered that a leaf area of about 1·0–1·2 sq.mm. is the minimum area that can be presented to individual insects.

It was thought that humidity conditions and period of starvation prior to feeding might be important. Starvation, for example, might increase the voracity of the larva—resulting in a greater area of leaf being consumed during the interval between the beginning of feeding and the time when sufficient insecticide has been absorbed by the insect to cause inhibition of feeding. In a preliminary experiment batches of forty last-instar *P. maculipennis* larvae were starved for 3, 13 and 24 hr. at relative humidities of 0, 50 and 100% at 75° F. They were then fed individually with strips from young cabbage leaves, 2·2 sq.mm. in area, bearing a deposit of 0·00048 mg. lead arsenate per strip. Areas of leaf consumed were determined as before.

TABLE 5. *The effect of humidity conditions and starvation on the feeding of fourth-instar larvae of* P. maculipennis *on foliage treated with lead arsenate*

(Average weight of larvae = 3·86 mg.)

	Leaf area consumed (sq.mm.)	Percentage kill
Period of starvation: 3 hr.	1·30	40·0
13 hr.	1·13	45·9
25 hr.	1·37	49·8
Relative humidity: 0%	1·14	47·2
50%	1·20	48·0
100%	1·40	43·7

Table 5 shows the summarized results. No increase in leaf area consumed after long periods of starvation is apparent and present indications are that 2 hr. starvation is adequate and 100% relative humidity is the most satisfactory for the fourth-instar larvae of *P. maculipennis*. The kills recorded substantiate these conclusions since it appears that both low humidities and long periods of starvation cause some decrease in resistance. A more detailed study of the effect of pre-treatment environment on the susceptibility of mandibulate insects to stomach poisons would be of interest.

Feeding experiments using different leaves showed that for any one species of food plant the thinner and younger the leaf the greater is the area consumed in a given period of time. A study of different food plants of *P. maculipennis* showed

that turnip leaves, from plants grown in the glasshouse, were more quickly consumed than were leaves and cotyledons of various cabbage and brussels sprouts varieties, and leaves of mustard.

Thus about 90% of the larvae of *P. maculipennis* weighing 3·2–4·0 mg. will consume completely areas of turnip leaf of 1·2–1·4 sq.mm. bearing a lethal dose of lead arsenate. Data from five experiments in Table 7 show the percentage of leaf strips completely consumed to be 96, 93, 90, 87 and 96 in successive experiments.

Unfortunately, the behaviour of this insect is not so satisfactory when presented with other insecticides. Only about 10% of larvae completely consumed leaf strips 1·2 sq.mm. in area bearing dosages of rotenone as great as 0·0018 mg./strip. Feeding generally ceased within a minute or two and paralysis of the larvae set in within 5 to 10 min., but within 8 hr. there was complete recovery—the larvae feeding voraciously on fresh food and pupating normally. Similarly with γ-hexachlorocyclohexane (γ-B.H.C.) and with D.D.T. larvae ceased to feed after a short time. Treated leaf strips 1·2 sq.mm. in area were completely eaten by about 60% of larvae although the lethal dose of these insecticides was readily consumed. With such insecticides determination of the leaf areas eaten and the dose of insecticide consumed by individual larvae is necessary. Details of the analysis of such data are given later.

Larvae of some of the larger Lepidoptera particularly in the later instars will consume completely larger areas of leaf without signs of being checked by such insecticides as lead arsenate, rotenone, γ-hexachlorocyclohexane, chloromethyl chlorophenyl sulphone and E605. Thus last-instar larvae of *Diataraxia oleracea*, *Phlogophera meticulosa* and *Barathra brassicae* will consume completely treated leaf areas of 50 sq.mm. or greater, but with D.D.T. the high speed of action may again prevent the larvae from feeding before the whole area has been consumed.

TABLE 6. *Feeding of last-instar larvae of* D. oleracea *on strips of cabbage foliage 48 sq.mm. in area treated with different concentrations of D.D.T.* (16 Oct. 1946)

Average weight of larvae (g.)	Dose per larva (mg.) on 48 sq.mm.	No. of insects	No. of strips not completely consumed	Average area leaf strip consumed (sq.mm.)	Percentage kill
0·33	0·012	12	6	39·2	58
	0·024	11	3	40·9	100
	0·036	8	4	38·8	88
0·45	0·012	11	6	38·7	9
	0·024	10	4	41·9	10
	0·036	12	5	37·7	25
	0·042	9	3	40·2	89
0·55	0·024	8	2	46·0	0
	0·036	8	3	41·2	0
	0·042	14	2	47·3	43

Table 6 shows data from an experiment in which last-instar larvae of *Diataraxia oleracea* of various weights were fed with leaf strips, 48·4 sq.mm. in area, bearing different weights of D.D.T. deposit.

Where strips were not completely eaten the areas consumed were calculated by measurement of the partially eaten leaf strips over millimetre graph paper using a low power dissecting microscope.

The results in Table 6 suggest that with this insect, for any one larval weight, a fairly constant area of leaf strip is consumed before the larva ceases to feed, irrespective of size of dose. The picture is, however, somewhat complicated since a dose on 48·4 sq.mm., just sublethal for the smallest larvae (0·2 g. in weight), is generally completely consumed, whereas, with the largest larvae the dose, which is just sublethal, is higher and apparently present in a quantity sufficient to cause inhibition of feeding in as short a period of time as a lethal dose.

Thus within the sublethal range there appears to be a threshold weight of deposit which, if exceeded, causes cessation of feeding, irrespective of the size of the larva, though with increase in larval weight there is some increase in area of leaf consumed before feeding ceases.

Details of apparatus and methods used for feeding treated foliage

Of the methods that have been used two general techniques are described in detail, the first applicable to small larvae such as those of *Plutella maculipennis* and *Phaedon cochleariae* and the second for larger insects, particularly the later-instar larvae of various Lepidoptera.

Removal of leaf strips from treated foliage

The foliage is first treated with a given weight of poison applied to the leaves by means of the spraying tower described.

Strips are removed by means of the cutting tools shown in Pl. 4 (*c*) and (*d*). A tool consisting of a pair of dividers with knife-edged tips is used for cutting large leaf strips 5 mm. or more wide (Pl. 4, *c*) and for the smallest leaf strips, 1–5 mm. wide, the tool shown in Pl. 4 (*d*) is used.

It is essential that the knife edges of the tools should be very sharp and that the leaf areas to be cut should be fully turgid otherwise distortion is liable to occur.

The principle of the small feeding apparatus is as follows: The animal placed in the wide part of the chamber (Text-fig. 4, CH_1) soon finds its way down the channel (CH_2) along which it can crawl comfortably until the leaf strip is reached. This forms a horizontal strip blocking the passage of the animal above and below and consequently the body of the insect cannot come into contact with the treated upper surface. Contact effect on the body is prevented in this way, so that the insect is forced to eat the leaf strip from its edges and only the head can come into contact with the treated surfaces. There is thus still a danger of the insecticide acting as a contact poison on the mouthparts, but except by a method of direct injection into the gut such action cannot be prevented.

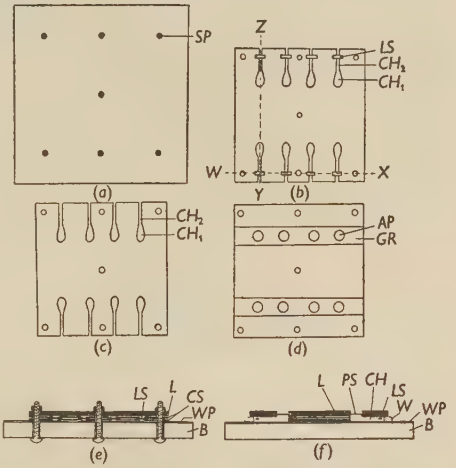
The insects are removed $\frac{1}{2}$–1 hr. after they have been placed in the feeding apparatus. When the leaf strips have not been completely consumed, a pad of damp

filter paper is then introduced beneath the lid of the apparatus. This maintains the partially eaten leaf strips fresh and turgid until the areas consumed can be calculated.

Insects after removal are placed individually in numbered cages on trays one of which is shown in Pl. 4 (*f*). Each cage consists of a glass ring, approximately 1·5 cm. diam. and 1·5 cm. in height with a lid consisting of butter muslin glued to a 2·5 cm. diam. galvanized iron curtain ring. The tray bears a shallow layer of wet sand covered by a sheet of glass which is slotted with slit-like apertures 1·3 cm. long and 2–3 cm. apart. Into each slot is slipped a circle of fresh leaf 1·5 cm. diam. one edge of which is pushed into the wet sand, thus maintaining the leaf in good condition for consumption by those insects which may recover from the effect of the insecticide. One cage enclosing a single insect is placed over each of the projecting leaf circles.

The above technique, whereby the insect is enclosed in a channel, is not suitable for the larger insects, many of which are irritated by the confined space, and the apparatus finally adopted is shown in Pl. 4 (*e*) and (*g*). Leaf strips cut with the tool shown in Pl. 4 (*c*) are fitted in holders (Text-fig. 5 *a*) in which a thin strip of celluloid acts as a shield to the treated surface and prevents the body of the insect from being affected by any contact action of the insecticide.

As previously mentioned the above techniques are not satisfactory for feeding stomach insecticides to *all* species of mandibulate insects. Among Lepidoptera a feeding apparatus which has proved satisfactory in preliminary trials with winter moth larvae is shown in Text-fig. 5 *b*. A modification of this has



Text-fig. 4. *a–d*, surface views of individual parts of feeding apparatus. *a*, base; *b*, lower celluloid sheet, 3 in. square, 0·079 cm. thick; *c*, upper celluloid sheet, 3 in. square, 0·053 cm. thick; *d*, lid. *e–f*, diagrammatic sections through positions *W–X* and *Y–Z* respectively of complete apparatus. *AP*, aperture; *B*, base of 10 cm. square plywood through which pass seven 6 BA ½ in. bolts; *CH*, channels cut in celluloid sheets (*CS*) consisting of an outer part (CH_2) about 1·25 cm. long and 0·1143 ± 0·00025 cm. wide (75° F.) connecting with an inner part (CH_1) about 0·5 cm. maximum width; *GR*, groove in lid (*L*) through which filter paper strip (*PS*) is slid to close apertures (*AP*) and enclose each insect in its channel; *L*, lid drilled with eight 0·5 cm. diameter holes through which insects are dropped into the channels (CH_1) beneath; *LS*, leaf strip with sprayed surface uppermost resting on lower celluloid sheet across channel (CH_2) and held in position by upper celluloid sheet; *SP*, projecting ends of bolts in base to which other parts of the apparatus are fitted and secured; *W*, wick of damp cotton-wool which maintains leaf strip fresh and turgid and prevents escape of test insect after consumption of leaf strip; *WP*, waxed paper sheet forming floor of insect chambers which is renewed before each experiment.

been moderately successful with adult mustard beetles, *Phaedon cochleariae*, though detailed toxicological trials have not been carried out. As yet, no suitable techique has been devised for a geometrid larva, *Xantheroe fluctuata*, a species easily reared in the laboratory. Small numbers of adults and larvae of the beet carrion beetle, *Blitophaga opaca*, were available at one time, but the ragged method of feeding these insects rendered quantitative determinations of area of leaf consumed impossible. Early instar-larvae may only nibble at leaves and consume areas too small to be determined with any accuracy, while obvious practical difficulties prevent studies on such important insects as flea beetles.

No studies have been made on the Orthoptera.

Text-fig. 5. *a*, diagram of holder for feeding treated leaf areas to large lepidopterous larvae; *b*, diagrammatic section of feeding apparatus for winter moth larvae. *B*, base of 0·5 cm. thick cork; *CP*, pad of moist cotton-wool; *CS*, celluloid shield; *GR*, 0·5 cm. diameter glass ring; *IC*, insect chamber; *IGT*, 3 cm. length of 0·48 cm. glass tubing enclosing the insect chamber; *LC*, treated leaf circle; *LS*, treated leaf strip; *M*, muslin; *OGT*, glass tubing which holds part of the apparatus in position; *P*, pin holding celluloid shield and leaf strip to base; *RT*, rubber tubing which holds parts of the apparatus together.

Brief mention should be made of some quantitative techniques used for determinations of combined contact and stomach poison effects of insecticides and also for studies on cessation of feeding. One method is described in detail in another paper (Way & Stoker, 1949*b*). The other involves the use of the apparatus shown in Pl. 4 (*f*). As described already this consists of a series of small cages each enclosing a leaf circle with one edge dipping into damp sand. Such circles with both surfaces previously covered with known deposits of insecticide by means of the spraying apparatus are enclosed with one or more test insects. Records are made at intervals of the condition of the insects and extent of feeding which can be determined by placing the partly eaten circles over millimetre graph paper.

Examples of experimental data obtained in stomach poison trials

Certain data obtained in five separate experiments on the determination of the toxicity of acid lead arsenate as a stomach poison to fourth-instar larvae of *Plutella maculipennis* will be described in detail since it indicates the efficiency of the technique and illustrates some of the problems involved in quantitative laboratory assessments of toxicity.

Essential details are given below, the same procedure being followed in all experiments.

Details of spraying

Insecticide: 2% w/v lead arsenate powder (98–99% pure acid lead arsenate) suspended in distilled water. Particle size about 5 μ.

Details of spray deposition: volume sprayed per application, 5 c.c.; time taken to spray, 5 sec.; air pressure, 45–50 lb./sq.in.; time with false bottom in position, 30 sec.; times allowed for depositions of insecticide, 25 or 35 sec.; no. of sprayings per total deposit, 4–8 with various combinations of above deposition times.

The foliage sprayed consisted of the distal portions of leaf laminae of small turnip plants grown in pots in the glasshouse.

Rearing of test subjects

This was carried out in the glasshouse using a technique described by Way & Stoker (1949*a*). Young larval instars were fed on potted turnip plants grown in the glasshouse and third-instar larvae transferred either to January King cabbage grown outdoors and potted just prior to the onset of hearting, or to similarly treated brussels sprouts plants.

Treatment of test subjects

Fourth-instar larvae of a constant weight (error = $\pm$ 0·2 mg.) were selected. Such larvae were starved for 8–10 hr. at 75° F. and 100% R.H. They were then divided at random into batches of thirty to forty and individuals of each batch fed with strips of foliage 1·20 sq.mm. in area bearing a particular deposit of arsenate. One batch was fed with each of the dosages obtained as described above and a further control batch was fed with strips of untreated leaf.

The apparatus was kept in a constant temperature cabinet at 75° F.; feeding was generally complete within 10 min. and after $\frac{1}{2}$ hr. individual larvae were transferred to cages with fresh food.

Three days later larvae could be readily divided into those dead and those normal. In no case did a larva which had consumed a fatal dose eat any of the fresh food provided: a useful observation since by this as well as by other means, larvae which die through other causes can be distinguished. This has been observed with other insecticides used—notably D.D.T. and γ-B.H.C.—except that with these insecticides a certain amount of abnormal nibbling may be carried out by fatally poisoned larvae.

Results obtained in the series of five experiments with fourth-instar larvae of *P. maculipennis* and lead arsenate are shown in Table 7 (*a–e*).

Probit analysis of the data is represented graphically in Text-fig. 6 and essential details shown in Tables 8 and 9.

Comparison of the L.D. 50's in Table 8 shows an insignificant decrease in resistance from 10 to 11 April 1946. Resistance was apparently slightly lower on 19 August 1946, but a marked significant decrease was apparent on 30 September 1946 and 2 October 1946. Many factors may have been responsible for this. The insects were not reared under constant temperature and lighting conditions and

TABLE 7. *Toxicity of acid lead arsenate as a stomach poison to fourth-instar* P. maculipennis *larvae showing the variation in results obtained on five different dates*

	Dose per larva $\times 10^5$ (mg.)	No. of insects used	Percentage kill	Notes
(*a*)	Control	32	0	Date: 10 April 1946
	29	38	16	Original no. of larvae per batch, 40
	36	37	60	(Control 32)
	40	40	73	Average weight of larvae, 3·7 mg.
	42	39	74	
	45	38	84	
	52	39	92	
(*b*)	Control	30	0	Date: 11 April 1946
	32	39	44	Original no. of larvae per batch, 40
	36	39	62	(Control 30)
	39	35	77	Average weight of larvae, 3·65 mg.
	41	36	80	
	44	39	87	
	45	36	86	
	50	37	95	
(*c*)	Control	32	0	Date: 19 August 1946
	30	36	31	Original no. of larvae per batch, 40
	35	37	70	(Control 32)
	37	35	83	Average weight of larvae, 4·0 mg.
	40	36	89	
	44	33	91	
	49	40	100	
(*d*)	Control	28	0	Date: 30 September 1946
	20	33	6	Original no. of larvae per batch, 40
	24	37	24	(Control 28)
	28	35	66	Average weight of larvae, 3·6 mg.
	32	38	79	
	36	31	93	
(*e*)	Control	32	0	Date: 2 October 1946
	20	31	13	Original no. of larvae per batch, 32
	24	30	23	except *=24.
	28	31	61	Average weight of larvae, 3·8 mg.
	32	32	75	
	36	22*	86	

All larvae which failed to consume their full dose were discarded, but in later experiments they were kept and those which died were included in the analysis of results. Individuals which lived were discarded if their number was less than 5 % of the total.

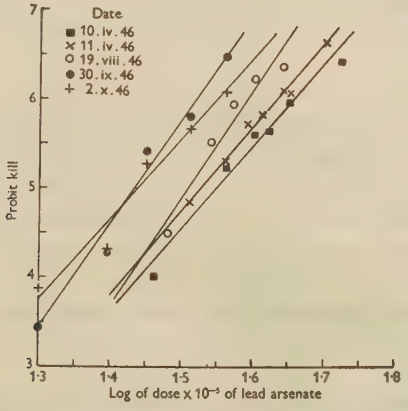
Methods are described later in which all individuals of a batch are included in the results irrespective of leaf area consumed.

TABLE 8. *Probit analysis of experiments using acid lead arsenate as a stomach poison for fourth-instar* P. maculipennis *larvae*

Date	Slope (*b*)	χ^2	Hetero-geneity	L.D. 50/larvae $\times 10^5$ (mg.)	Fiducial limits of L.D. for 5 % probability level
10 Apr. 1946	9·44 ± 1·30	1·84 (4 d.f.)	None	35·3	34·3–36·2
11 Apr. 1946	9·56 ± 1·62	0·53 (5 d.f.)	,,	33·5	32·6–34·5
19 Aug. 1946	13·15 ± 1·87	2·50 (4 d.f.)	,,	32·5	31·7–33·3
30 Sept. 1946	11·66 ± 1·42	1·19 (3 d.f.)	,,	27·0	26·5–27·6
2 Oct. 1946	9·10 ± 1·43	1·41 (3 d.f.)	,,	27·3	26·5–28·1

d.f. = nos. of degrees of freedom.

variation in climatic factors may have been responsible. However, a further factor is involved which indicates the importance of keeping a detailed record of rearing and experimental conditions. Larvae for the first three experiments were reared on January King cabbage, the foliage of which is rather tough. For the latter two experiments brussels sprout was used as food plant, the leaves of which were relatively 'lush' and it was noted that larvae reared on these plants grew to a larger size than those reared on January King. Thus larvae of a particular size (i.e. weight) would be in a relatively earlier stage of development if selected from brussels sprouts than from January King. It is suggested that this factor may be one of those affecting the resistance of the test insect. Clearly if reproducible results are to be obtained standardizations of rearing as well as standardization of experimental technique is required (Way & Stoker, 1949*a*). The production of an insect popu-



Text-fig. 6. Graphs showing data obtained on the variation of resistance of fourth-instar *Plutella maculipennis* larvae to acid lead arsenate as a stomach poison, using the technique described.

lation of a constant average resistance level is more important in a stomach poison technique than in contact insecticide trials, since in the former the individual treatment required limits the number of insects that can be treated at any one time. Thus, if data obtained in one day are to be compared with those obtained on a succeeding day, it is essential that the level of resistance of the insect test-subjects should remain consistent. Further reference will be made to the problem in connexion with experimental data described later in the paper.

Although all five probit regression lines show a positive test for parallelism the experiments on 30 September 1946 and 2 October 1946 are worthy of mention in view of the apparent difference in slope of the probit regression lines in the 2 days. Table 9 shows the L.D. 10's and 90's on the 2 days and indicates a lower L.D. 10 and a higher L.D. 90 on the latter day.

An examination of the recorded details of the experiments showed that on

30 September 1946 larvae were carefully selected according to size whilst on 2 October 1946 the number of individual test insects available were relatively few and such careful selection could not be made. Consequently, although the average weight was similar the variation about the mean weight was greater, and since larval weight affects susceptibility there would be greater individual variation in resistance to the insecticide on 2 October 1946. As a result the slope of the probit regression curve would tend to be less steep. Further reference to the problem of larval body weight will be made later.

TABLE 9. *Calculated* L.D.*'s for* 10 *and* 90% *kills in experiments on* 30 *September* 1946 *and* 2 *October* 1946 *using acid lead arsenate as a stomach poison for* P. maculipennis *larvae*

Date	L.D. 10/larva $\times 10^6$ (mg.)	Fiducial limits of L.D. 10 for 5% probability level	L.D. 90/larva $\times 10^6$ (mg.)	Fiducial limits of L.D. 90 for 5% probability level
30 Sept. 1946	21·0	20·2–21·9	34·8	33·9–35·8
2 Oct. 1946	19·7	18·6–20·9	37·7	35·6–40·1

As previously mentioned, with some insecticides speed of action is such that small insects, particularly, may consume only a portion of the leaf area presented. Variation in area of the portion consumed may also be considerable and thus similar treatment of a number of individuals as in the previous experiment is impossible. However, if the leaf area eaten and dose consumed per larva are determined, the results may be analysed in two ways.

(i) The average dose consumed per treatment calculated.

(ii) Larvae grouped according to dose of insecticide consumed irrespective of original dose presented.

Results of an experiment with lead arsenate as a stomach poison for fourth-instar *P. maculipennis* larvae illustrate these methods of presentation. Batches of forty larvae of average weight 3·5 mg. were fed with four doses of acid lead arsenate powder on fairly tough leaf strips on Winnigstat cabbage, 2·2 sq.mm. in area. Such leaf strips were rarely completely consumed. Leaf areas eaten per larva were determined by means of a calibrated squared micrometer eyepiece in a dissecting microscope. Thence doses consumed were calculated. For the second method of presentation larvae were grouped into four batches according to dosage consumed, the average doses being 20, 35, 50 and 65 mg. $\times 10^{-5}$/larva.

Summarized results are shown in Table 10.

Results of probit analysis of the two sets of data are shown in Table 11.

Examination of the fiducial limits in Table 11 shows that the difference in L.D. 50 obtained by the two methods is insignificant, but the difference in slope of the regression lines is such that they do not satisfy the test for parallelism. It is considered that errors in method I are the main cause for the difference, since a method, whereby average dose per batch is determined, would be expected to be in error at low and high levels of kill.

TABLE 10. *Two methods of presentation of data obtained by feeding fourth-instar* P. maculipennis *larvae with acid lead arsenate on leaf strips, 2·2 sq.mm. in area. (Date: 1 June 1945)*

Weight of lead arsenate on 2·2 sq.mm. × 10^5 (mg.)	Method I			Method II	
	No. of larvae per treatment	Average dose consumed × 10^5 (mg.)	Percentage kill	Average dose consumed × 10^5 (mg.)	Percentage kill
43	38	28	34	20	8
62	40	38	63	35	53
80	36	43	70	50	94
87	31	58	77	65	96
Control	34	—	0	—	—

TABLE 11. *Probit analysis of data from Table 10 showing comparison of* L.D. 50*'s obtained by two methods of presentation of results*

Method	Slope (b)	χ^2	Hetero-geneity	L.D. 50/larva × 10^5 (mg.)	Fiducial limits of L.D. 50 for 5% probability level
I	$3·91 \pm 1·02$	1·02 (2 d.f.)	None	34·0	31·6–36·6
II	$6·83 \pm 1·07$	1·18 (2 d.f.)	None	33·0	31·5–34·5

Method II does not take into account certain other factors which may be concerned in stomach poisoning, notably the effect of the amount of leaf area consumed. Poisoning would be expected to be more acute when the dose is presented on a small area of leaf which is rapidly consumed than when it is distributed on a larger leaf area. A larger leaf area may also have the effect of diluting the insecticide and slowing down its absorption by the gut. Such factors show some effect in the present experiments, since with both lead arsenate and D.D.T. small increases in kill occurred with increase in concentration of the dose on the leaf area. However, the increases were of low significance and the importance of these factors is being made the subject of further investigation.

Experiments with D.D.T. as a stomach poison for fourth-instar larvae of *P. maculipennis* were carried out in some detail. In one experiment, carried out on two consecutive days, batches of thirty to forty larvae were fed with each of ten doses of D.D.T. deposited from acetone solution on leaves of young Harbinger cabbage. Leaf strips 2·2 sq.mm. in area were presented to individual larvae and the areas eaten and dosages consumed calculated as before.

Further details of treatment are: average weight of larvae, 3·8 mg.; period of starvation, 6–9 hr.; temperature of experiment, 75° F.

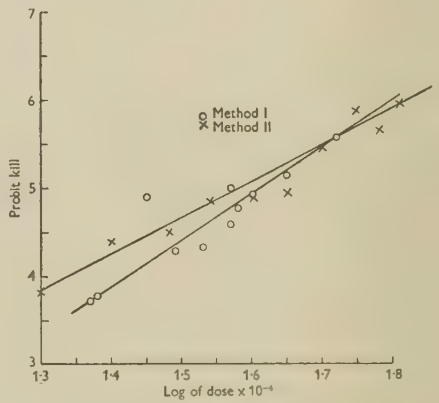
Results of the two methods of presentation of data are shown in Tables 12 and 13 and Text-fig. 7.

With this insecticide the regression lines of the two methods of analysis show parallelism in all experiments. A possible cause of this is that the slope of the regression curve in method I is made more steep as a result of contact action of

D.D.T. on the mouthparts of the insect which would be greatest at the higher concentrations. In method II the effect of contact action would be more uniformly distributed at all points on the regression line. However, these suggestions should be treated with reserve until more is known about the extent of contact action which can occur through the mouthparts.

TABLE 12. *Two methods of presentation of data obtained by feeding fourth-instar* P. maculipennis *larvae with D.D.T. on leaf strips 2·2 sq.mm. in area. (Date 20–21 October 1945)*

Weight of D.D.T. on 2·2 sq.mm. $\times 10^8$ (mg.)	Method I			Method II	
	No. of larvae per treatment	Average dose consumed $\times 10^6$ (mg.)	Percentage kill	Average dose consumed $\times 10^6$ (mg.)	Percentage kill
61	39	23	10	20	12
61	37	24	11	25	27
73	35	28	46	30	31
61	33	31	24	35	45
49	32	34	25	40	46
73	32	37	34	45	48
73	26	37	50	45	72
73	34	38	41	55	81
61	34	40	47	60	75
85	27	45	56	65	83
97	36	53	72	—	—



Text-fig. 7. Graphs showing comparison of data obtained by two different methods of presentation of results of an experiment in which D.D.T. was fed to fourth-instar *Plutella maculipennis* larvae on leaf strips which were not completely consumed.

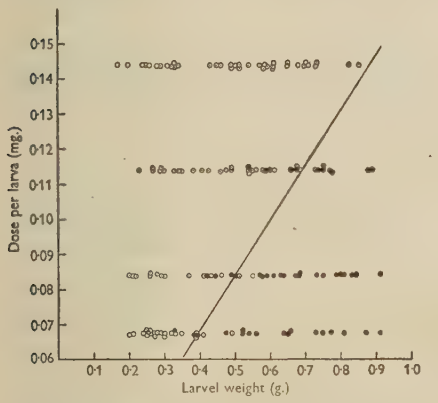
TABLE 13. *Probit analysis of data from Table 12 showing comparison of* L.D. 50*'s obtained from two methods of presentation of results*

Method	Slope (b)	χ^2	Hetero-geneity	L.D. 50/larva $\times 10^6$ (mg.)	Fiducial limits of L.D. 50 for 5% probability level
I	$5{\cdot}25 \pm 0{\cdot}72$	15·8 (9 d.f.)	None	41·0	39·3–42·9
II	$3{\cdot}99 \pm 0{\cdot}51$	4·0 (8 d.f.)	None	38·6	37·0–40·3

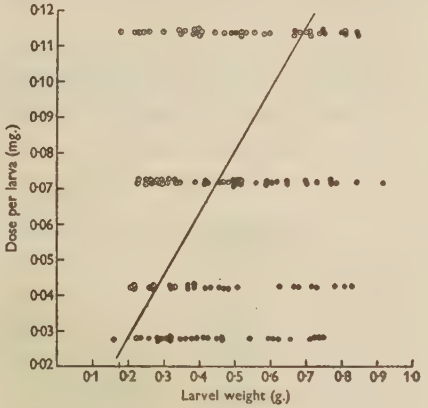
Again no significant difference in L.D. 50 is noticeable. The low χ^2 value in method II indicates this to be the more satisfactory method of analysis.

The effect of body weight on insect susceptibility

Reference has already been made to the problem of body weight and its effect on insect resistance. Within a single instar, lepidopterous larvae may increase in weight by four times or more during development and previous workers have in many cases assumed that, within the instar, weight increase is directly proportional to increase in insect resistance. On this assumption dosage has been expressed in terms of body weight of larva. For example, the M.L.D. has been defined as the dose in mg./g. of body weight required to kill 50% of individuals of a particular insect species. Data



Text-fig. 8. (Exp.: 9 Oct. 1946.) The effect of four doses of acid lead arsenate on fifth-instar larvae of *Phlogophera meticulosa* of varying body weights. ⊙ = insects which die; ● = insects which recover or are unaffected.



Text-fig. 9. (Exp.: 11 Oct. 1946.) The effect of four doses of acid lead arsenate on fifth instar larvae of *Phlogophera meticulosa* of varying body weights. ⊙ = insects which die; ● = insects which recover or are unaffected.

to be published later will show that this assumption is in many cases not warranted, but in the present paper it is intended only to give one example to show the extent of importance of the body weight factor on susceptibility of lepidopterous larvae. This experiment was carried out twice and both sets of results are described in detail since they give some idea of the accuracy of the technique employed.

In each experiment about 160 fifth-instar larvae of *Phlogophora meticulosa*, the angleshades moth, were selected from the rearing cage, starved for 9–10 hr. at 75° F.,* weighed individually and divided at random into four batches of 30–40 individuals per batch.

A suspension of 7% w/v lead arsenate powder (98–99% pure acid lead arsenate) of particle size about 5 μ was used to deposit four dosages of arsenate on foliage of

* Larvae of this species are nocturnal feeders and during much of the period of 'starvation' mentioned above would normally not be feeding.

Winnigstat cabbage. Strips 48·4 sq.mm. in area were cut from the treated foliage and fed to individual larvae by means of the method previously described, each batch of larvae being fed with one of the four dosages obtained. Fresh food was provided when the dose of insecticide had been consumed. The experiment was carried out in a constant temperature cabinet at 75° F.

Text-figs. 8 and 9 show in graphical form the results obtained. The curve in each case is the calculated line representing the increase in L.D. 50 as the larva increases in weight. Table 14 shows the summarized results in which each batch of larvae has been subdivided into three weight groups: (1) larvae weighing 0·20–0·33 g.; (2) larvae weighing 0·34–0·54 g.; (3) larvae weighing 0·55–0·91 g. with average weights of 0·26, 0·43 and 0·71 g.

TABLE 14. *Toxicity of acid lead arsenate as a stomach poison to fifth-instar larvae of* P. meticulosa, *showing the percentage kills of larvae of three different weight groups caused by four dosages of insecticide*

		Mean larval weights × 10^1 (g.)					
		2·6		4·3		7·1	
Date	Dose/larva × 10^2 (mg.)	No. of insects	Percentage kill	No. of insects	Percentage kill	No. of insects	Percentage kill
9 Oct. 1946	6·7	17	94	11	45	10	0
	8·4	8	100	9	55	19	5
	11·4	7	100	12	92	17	41
	14·4	9	100	10	100	17	94
11 Oct. 1946	2·8	10	20	9	0	8	0
	4·2	9	67	10	0	8	0
	7·1	15	100	17	41	15	0
	11·3	6	100	16	94	17	59

The statistical analysis of the results followed lines similar to those described by Finney (1943) in his paper on the assessment of potency which depends on two factors, and by Bliss (1938), who described the analysis of probit data when the number of insects tested at each level of dosage may be small.

In order to find the nature of the relationship between percentage mortality, dose and weight of larvae, the empirical probit corresponding to the percentage killed was plotted against log-dose (x_2) for each weight group. This plotting indicated that the probit was linearly related to dose for each weight group and that the lines for each were parallel with a slope of b_2. Similarly it was found that the empirical probits were linearly related to logarithm of weight of insect (x_1) for each value of the dose, the lines for each dose being parallel with slope b_1. It was therefore decided to fit probit planes of the form

$$Y = a + b_1 x_1 + b_2 x_2,$$

where $x_1 = \log \text{weight} + 1$ (g.) and $x_2 = \log \text{dose} + 2$ (mg.).

The procedure for fitting a plane of this form has been given by Finney (1943, 1947).

Estimates of b_1 and b_2, b_1o and b_2o were obtained from the plotting described above. The quantity a was estimated by considering, for the various combinations of weight of insect and logarithm of dose, the quantities

$$(\text{empirical probit} - b_1o \log \text{weight} - b_2o \log \text{dose}),$$

which were approximately constant.

A provisional plane having been obtained, the usual procedure was followed, namely, to obtain provisional probits for each combination of dose and weight, then working probits and weights and so obtain a new provisional plane. This process was continued as for the fitting of a probit line, until the plane obtained by the calculations differed inappreciably from the provisional plane on which it was based; that is, until the regression coefficients of the fitted plane did not differ appreciably from those of the provisional plane compared with their standard errors. Finally, as Finney showed in this paper (1943), standard errors for the coefficients of the regression equation were obtained and the quantity χ^2 evaluated, in order to test whether the hypothesis of the probit plane in the particular form chosen satisfactorily represented the data. The results of analysis of the above experiments are shown in Table 15.

TABLE 15. *Probit analysis results of two experiments to determine the effect of body weight on susceptibility of fifth-instar* P. meticulosa *larvae to lead arsenate*

Date	a	b_1	b_2	χ^2	χ^2 required for 5% probability level	Hetero-geneity
9 Oct. 1946	1·90	$-9·77 \pm 1·86$	$10·69 \pm 2·13$	5·9 (5 d.f.)	11·1	None
11 Oct. 1946	3·78	$-8·47 \pm 1·27$	$7·87 \pm 1·12$	5·8 (6 d.f.)	12·6	None

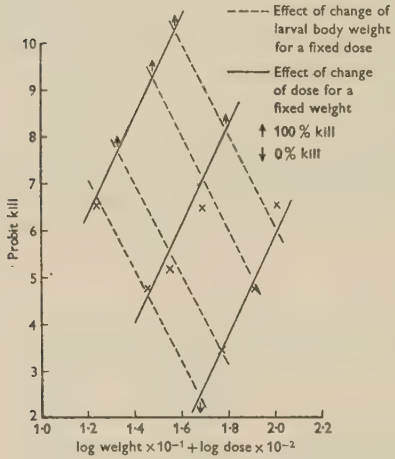
Since the values of the χ^2's are well within the bounds of random variation it may be said that the probit plane provides a satisfactory description of the experimental results. Planes for the two experiments are represented graphically in Text-figs. 10 and 11. Table 16 shows the estimates of the L.D. 50 for the three larval weight groups in each experiment.

TABLE 16. *Values of the* L.D. 50 *of lead arsenate as a stomach poison for fifth-instar larvae of* P. meticulosa *of three different body weights*

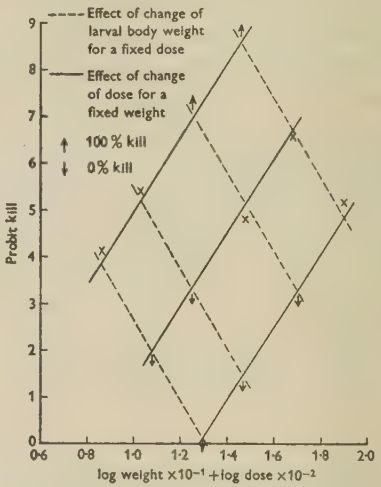
	Larval weight (g.)					
	0·26		0·43		0·71	
Date	L.D. 50 (mg.)/larva	Fiducial limits for 5% probability level	L.D. 50 (mg.)/larva	Fiducial limits for 5% probability level	L.D. 50 (mg.)/larva	Fiducial limits for 5% probability level
9 Oct. 1946	0·047	0·042–0·052	0·074	0·070–0·077	0·117	0·112–0·122
11 Oct. 1946	0·039	0·036–0·042	0·068	0·065–0·071	0·117	0·110–0·125

The ratio of weight increase to dose increase required to give 50% kill is seen to be constant. Values were 1 : 0·93 on 9 October 1946 and 1 : 1·05 on 11 October 1946

and, in fact, it would appear that with the fifth instar of this insect there is a simple 1:1 relationship between weight and dose when lead arsenate is used as a stomach poison. Since, as in this experiment, larval weights can be of equal importance to dose it is essential that the former should be taken into account whenever determinations of the potency of stomach insecticides to lepidopterous larvae are carried out. Either larvae of a uniform weight must be selected, or body weight treated as a factor independent of dose.


Effect of change of larval body weight for a fixed dose
Effect of change of dose for a fixed weight
100 % kill
0 % kill
Probit kill
log weight ×10⁻¹ + log dose ×10⁻²

Text-fig. 10. (Exp.: 9 Oct. 1946.) Graphical representations of probit planes obtained by analysis of results of an experiment in which the fifth instar larvae of *Phlogophera meticulosa* of different weight groups were fed with a range of four doses of acid lead arsenate.


Effect of change of larval body weight for a fixed dose
Effect of change of dose for a fixed weight
100 % kill
0 % kill
Probit kill
log weight ×10⁻¹ + log dose ×10⁻²

Text-fig. 11. (Exp.: 11 Oct. 1946.) Graphical representations of probit planes obtained by analysis of results of an experiment in which the fifth instar larvae of *Phlogophera meticulosa* of different weight groups were fed with a range of four doses of acid lead arsenate.

I wish to express my thanks to Dr Potter and Dr Tattersfield for much helpful advice and criticism and to Mr O. Kempthorne for his stimulating interest in certain of the problems and for the help that he has given in connexion with the statistical analysis of the results.

Miss R. I. Stoker and Miss J. M. Bathgate have given invaluable assistance in the experimental work and I should also like to thank Miss B. A. Hopkins for the trouble and care she has taken throughout over the tedious job of rearing the test insects, and Miss P. R. Alexander for typing and checking the manuscript.

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EXPLANATION OF PLATE 4

(*a*) Feeding apparatus for small larvae with lid removed showing fourth-instar of *Plutella maculipennis* in process of feeding. (*b*) Feeding apparatus with lid bolted into place and showing one paper strip partly withdrawn from over two insect chambers. (*c*) Tool for cutting relatively large leaf strips. (*d*) Tool for cutting small leaf strips: d_1, flat brass rod the thickness of which determines the width of leaf strip; d_2, side-view of complete tool. (*e*) Larva of *Diataraxia oleracea* with treated leaf strip in leaf holder and surrounded by 4 cm. diam. glass ring. (*f*) Cages used for enclosing small insects with fresh food leaf. (*g*) Cages used for enclosing large insects during and after treatment. Holders for treated leaf strip are shown and three cages contain circles of fresh food leaf which are added after consumption of the poison dose by the insect. Most of the cages are covered by muslin lids.

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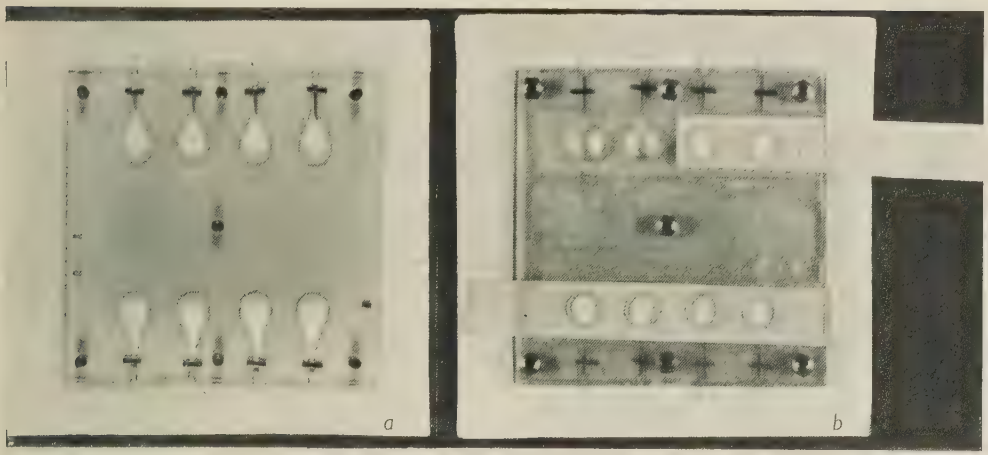

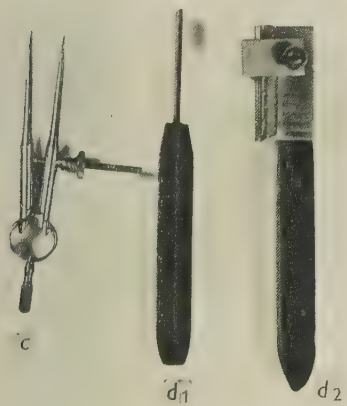

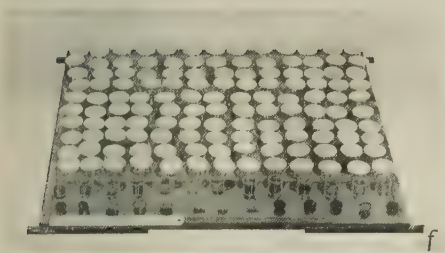

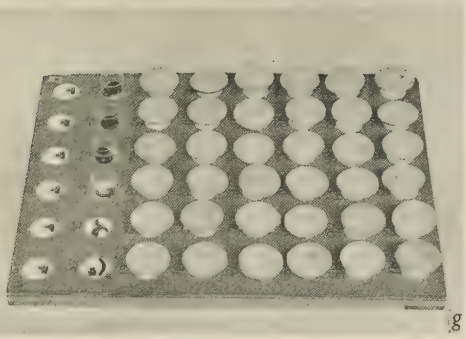

WAY—*Determining the stomach poison effect of insecticides*

THE EFFECT OF INSECTICIDES ON THE RESPIRATION OF *ORYZAEPHILUS SURINAMENSIS*: AN ATTEMPT TO COMPARE THE SPEEDS OF ACTION OF A NUMBER OF D.D.T. ANALOGUES*

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(With 17 Text-figures)

A method has been devised for observing the effects of a number of non-volatile contact poisons on the oxygen uptake of groups of *Oryzaephilus surinamensis*. Toxic concentrations of D.D.T. and of its analogues applied as dusts to *O. surinamensis* increase the rate of oxygen uptake: sublethal concentrations appear to have no effect. The total oxygen uptake of groups of starving *O. surinamensis* appears to be constant, whether or not they are treated with D.D.T. or its analogues. In each case a linear relationship appears between length of life and total volume respired before death.

The stimulus of D.D.T. to *O. surinamensis* is apparently quantal and the magnitude is independent of the concentration of D.D.T. in the dust. The stimuli resulting from the action of D.D.T. and its analogues are approximately equal, as are the rates at which the insects die. There is, however, apparently a correlation between molecular weight and length of life, i.e. there is a tendency for the insects treated with the poisons of lower molecular weight to die more rapidly than those treated with the analogues of high molecular weight.

The effect of benzene hexachloride on the respiration of *O. surinamensis* has been shown to be similar to that of D.D.T.

The effects of a number of insecticides on the oxygen uptake of *O. surinamensis* have been compared. As a result the insecticides have been classified in two groups: (*a*) those stimulating respiration, e.g. D.D.T., B.H.C., pyrethrins; (*b*) those depressing respiration, e.g. rotenone, Lethane B 71.

Introduction

The speed of action of insecticides

The speed of action of an insecticide has considerable importance not only practically but also from a theoretical aspect, since it is reasonable to expect that it will be dependent upon both site of action and physical properties of the poison. Thus in a series of poisons, such as the D.D.T. analogues, which can be expected to have a similar mode of action, the relative speeds of toxic action to one insect species are likely to be dependent solely upon those properties influencing the penetration of the poisons to the site of action. Information on the speed of action of a group of compounds, taken in conjunction with data obtained on the toxicity of the same

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group, may at least assist the interpretation of the toxicity data and afford some clue to the mechanism of toxic action.

The most obvious method of comparing the speed of action of a number of poisons is by making a series of visual inspections of the test subjects which have been given equivalent doses of the various poisons. This method of determining the speed of action of an insecticide such as D.D.T. is limited by the time taken to assess the condition of the test subject. This is especially so towards the end of the life of the insect and it is therefore difficult to examine repeatedly, at small and approximately equal time intervals, the large numbers of insects which are necessary to obtain a reasonably accurate estimate of the speed of toxic action of a poison by this method. Moreover, examination of the insects will not be without its effect on their physical condition due to both mechanical damage and environmental changes. In addition, there is considerable difficulty in assessing the conditions of insects suffering from D.D.T. poisoning, so that, although a precise estimate of affected and unaffected animals can be made it is difficult to differentiate the various stages ranking between normal and dead. Some other method of assessing the condition of the test subjects was therefore sought, preferably not one dependent on the fallible judgement of the operator.

The rate of respiration of an animal can be regarded as an index to its metabolism and can be used to assess its condition over a period of time; observations on respiration can be repeated at will without disturbing either the insect or its environment.

Choice of method

Of the methods used for the determination of respiration, those which involve chemical estimation of carbon dioxide output or oxygen uptake such as were used by Schäfer (1912), in his investigations on the mode of action of insecticides, are not really satisfactory for the continuous measurement of small rates of respiration in insects, unless very large numbers are used. Whilst such methods as those of Krogh (1916) are of use with larger animals they are likely to prove cumbersome and laborious when used with small insects. Manometric methods of observation of respiration are more sensitive and can be used to estimate the rate of respiration at intervals over a period of time. Preliminary experiments showed that the oxygen uptake of a reasonable number (twenty to fifty) of *O. surinamensis* could be measured in the usual Barcroft or Warburg respirometers (Dixon, 1934). Since the object of the experiments was to compare the speed of action of a number of poisons which would require a number of replicates, the use of a micro-respirometer for individual insects was not considered (Heatley, Berenblum & Chain, 1939).

As it was expected to make measurements over considerable periods of time the use of the Warburg type of respirometer was considered unsatisfactory since it is sensitive to small temperature changes in the constant-temperature bath and also to changes of atmospheric pressure. The Barcroft respirometer uses a compensating vessel and as a result of this it is not susceptible to atmospheric pressure

changes and is much less affected by slight variations in the temperature of the bath.

Finally, the Haldane type of respirometer in the form of a modification of the Barcroft apparatus (Dixon, 1934) was chosen. This type was used in preference to the Barcroft respirometer because the measurement of gas changes inside the apparatus is not dependent on the volume of the respiration chamber so that bodies of unknown volume may be introduced into the respirometer without affecting the accuracy of the readings. Moreover, the apparatus can be used in such a way that it has the same sensitivity as a Barcroft respirometer of the same dimensions.

Although D.D.T. takes a long time to kill, early experiments showed that only a short time elapses before it has an effect upon the insect treated. It was therefore considered necessary to commence taking observations as soon as possible after treatment with poison which considerably limited the methods available for its application. This fact, coupled with the use of a manometric technique for measuring respiration, limited the methods of application of the insecticide to those in which no volatile solvent is used, since it was desirable to administer it to the insect inside the container, and any trace of volatile solvent would disturb the manometers. If a volatile solvent were employed it would require 1–2 hr. before the insects could be placed in the respiration apparatus.

The test insects, *Oryzaephilus surinamensis*, are small and highly active and can climb on glass and thus, unless they are to be exposed to the danger of climbing into the caustic potash solution, used for absorbing the carbon dioxide produced in the apparatus, must be confined in some vessel. In addition, they require to be cooled to reduce their activity if it is to be possible to count a number of them into a vessel sufficiently small to be placed in the respiration apparatus. In the case of D.D.T. and its analogues which are not soluble in water and generally not very soluble in non-volatile solvents, only two methods of application of poison to *O. surinamensis* appear to be possible, a film technique and a dusting technique.

The employment of a film technique requires that the vessel holding the insects should be coated with poison before their introduction; variation in cooling the test subjects would cause differences in their activities and might thereby affect the rate at which the poison was picked up. This difficulty, arising from the need to reduce the activity of the test subjects in order to introduce them safely into the respiration chamber, can be avoided by using a dusting technique, for in this case the insects can be counted in a chilled state into a clean small vessel, and this can be kept at any desired temperature (in this case the temperature of bath for use with the respirometer) until they are treated with the poison dust. If the small vessel is closed by a piece of cloth, the poison dust can be introduced by brushing it through the cloth covering of the vessel. The vessel can then be introduced into the respirometer and readings commenced as soon as the apparatus has attained thermal equilibrium which then becomes the sole reason for delay in taking readings. By using an excess of dust, dosage effects due to the quantity of dust were eliminated.

Once the respirometers were set up it was found necessary for them to be in continuous operation for about a week. To prevent the blocking of the manometer tubes by the condensation of moisture, as a result of temperature differences, the whole apparatus was enclosed in a cellophane-covered framework and the air around the respirometers thus maintained at only one or two degrees below the temperature of the constant temperature bath.

General considerations

Data obtained by using the rate of oxygen uptake as an index of metabolism become more useful if it is possible to correlate them with the condition of the insects under consideration. Therefore, whenever a respiration experiment was discontinued the condition of the insects was assessed visually, so that it could be compared with the rate of oxygen uptake of the insects. This method of approach proved useful for the control tests, but it was necessary to carry out a special experiment in order to correlate the condition of D.D.T.-poisoned insects with their respiration.

From *a priori* reasoning, comparison of the speed of toxic action of different substances should only be made at equivalent dosage levels. Little information, however, is available on the relationship between dosage level and concentration of poison in dusts, and therefore tests were carried out in order to determine the effect of concentration of poison upon the speed of action. The speed of toxic action appeared to be independent of concentration in the dust and it was therefore possible to make direct comparisons between the speeds of action of poisons at any toxic concentration.

The nature of the data obtained in the course of these experiments makes it impossible to compare the action of two insecticides in any but a qualitative fashion unless the information can be expressed in terms of one or two parameters. It has been possible to do this by fitting a curve to data relating the rate of oxygen uptake with the time after the treatment of test subjects with the poison. The curve actually fitted does not give a unique fit, and the grounds for using it are only those of expedience. The curve, however, is easily fitted and its values are tabulated; the integral of the curve is also tabulated and thus an estimate of the total amount respired can be made.

Range of substances tested

Although the main object of the experiment was the comparison of the speeds of action of D.D.T. and its analogues, a number of other insecticides were included. In general, the effects of these substances on insect respiration have been such that it has not been possible to treat the rate of oxygen uptake-time curves in the same way as those obtained when D.D.T. and its analogues were used. Frequently it has only been possible to compare or contrast the effect of the substances on insect respiration.

Experimental

Preparation of poison dusts

In comparing the action of various poisons on the respiration of *O. surinamensis* the poisons were employed as 5% dusts in stockalite (china clay) carrier. The dusts were prepared by weighing out appropriate amounts of poison and carrier. The weighed amount of poison was placed in a mortar with an approximately equal bulk taken from the weighed amount of carrier and the two were thoroughly ground together; the rest of the carrier was then added a small portion at a time, whilst the grinding continued. To ensure an even distribution of the poison throughout the dust, the mixture was tipped from the mortar when all the carrier had been added and then put back a small portion at a time whilst grinding continued.

Test subjects

The test subjects used throughout were adult *O. surinamensis* selected from cultures 4–5 weeks old. The insects were reared on rolled oats at 90° F. in an atmosphere maintained in a humid condition by pads of cotton-wool soaked in water.

Setting up of respiration apparatus

The insects were removed from the culture and counted into a specimen tube, so that the tube contained two or three more insects than were required for the test. Care was taken to choose insects in good condition. The specimen tube was then closed with a piece of muslin held in place by a rubber band: the insects were shaken down to the bottom by a sharp tap and the tube placed in a cold chamber formed by a beaker standing in ice water. Under normal laboratory conditions it was necessary to cool the insects in this way for 1 hr. before they were sufficiently immobilized to permit the counting of them into the small tube in which they were to be introduced into the respiration apparatus. The tube consisted of approximately 2 cm. cut from the bottom of a $\frac{5}{8}$ in. specimen tube. As soon as the insects had been counted into the tube it was closed by placing a piece of bolting silk over its mouth and forcing into it a small cork ring to hold the silk in position (see Fig. 1*a*). The cork ring was cut from a cork fitting the tube.

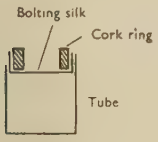

Fig. 1*a*.

Fig. 1*b*.

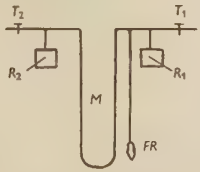

Fig. 1*c*.

By choosing a cork of the correct size and taking care in pushing it home, it was possible to confine the insects in such a way that they could not push between the bolting silk and glass and so avoid the poison dust when it was applied.

The bolting silk was then cut away flush with the top of the tube so that it would not become contaminated with caustic potash when the tube was placed in the respiration flask. The tube containing insects ready for the test was then placed in a box, in the constant temperature bath used for the manometers. The insects were allowed to remain in this box for some 2–3 hr., to allow them to become accustomed to the temperature conditions of the respiration vessel. The dust was introduced by placing it in the small cup formed by the cork ring and bolting silk, closing the tube, and gently brushing the dust so that it passed through

the bolting silk. The tube was then rotated slowly about its axis to ensure that each insect was covered with the dust and that the inside of the tube was completely dusted.

Just before the insects were dusted the ground-glass joints of the manometers were covered with a lubricant consisting of thirty parts vaseline and twenty parts beeswax, and small rolls of No. 40 Whatman filter paper were placed in the cups of the respiration and compensating flasks, and 0·4 ml. of 20% caustic potash solution were run on to each piece of filter paper. The compensating flash was then fixed to the manometer. When the insects had been dusted the tube containing them was placed carefully into the respiration flask which was then fitted to the manometer. Special care was taken not to jar the tube so that the film of dust adhering to the glass was not shaken off. The respirometer was then placed in the constant temperature bath and readings were commenced as soon as thermal equilibrium was attained.

Manometers

The manometers used were a modification of the Barcroft respirometer as described by Dixon (1934) except that the respiration vessels were made with specially wide necks to facilitate the introduction of solids (Text-fig. 1*b*).

The apparatus can be represented as in Fig. 1*c* where M is a manometer tube filled with kerosene coloured with a dye (Sudan III). T_1 and T_2 are taps opening into the atmosphere. R_1 is the respiration vessel and R_2 is a similar compensating vessel. Connected to the same side of M as R_1 is a graduated tube which contains mercury the level of which can be adjusted by means of the flexible reservoir FR, which can be compressed by means of a screw clip. R_1 and R_2 are attached to the respiration apparatus by means of the lubricated ground-glass joints.

Method of taking readings

Dixon (1934) in his description of the apparatus suggests that readings be taken by adjusting the mercury level in G, by compressing the reservoir FR, so that there is no pressure difference between the vessels R_1 and R_2 as shown by the manometer M. It is, however, possible to detect smaller changes in the movement of the manometer liquid than in the movement of the mercury level in G, so that small volume changes may be more accurately measured with the manometer than with the graduated tube. Therefore, at the beginning of each experiment, the manometer was calibrated in terms of volume changes by altering the mercury level in G and observing the pressure change indicated in R_1 by the manometer M. Subsequently, in the course of the experiment, manometer readings were taken prior to equating the pressures in R_1 and R_2 by adjusting the mercury level in G. In this way the manometer can be used to measure small volume changes more accurately than is possible with the graduated tube. The manometer is thus most useful for the measurement of small rates of oxygen uptake, whereas the graduated tube is of greatest use in making measurements involving relatively large volumes of oxygen, such as the total oxygen uptake of a group of insects.

The use of the manometer for the measurement of oxygen uptake is dependent on the stability of the mercury level in the graduated tube. It was found that the mercury level was sufficiently stable for this type of reading to be made providing time was allowed for the mercury column to settle down after any large change of volume (0·2 ml. or more) had been measured.

A full description of the method of using the respirometer is not given here as it is precisely the same as that used for the Barcroft respirometer, with the exception of the details set out above. In addition, in the experiments here described, the respirometers were not shaken since insects can be considered self-aerating.

Type of data obtained

Readings were commenced when the apparatus had finally attained thermal equilibrium, which was ½–1½ hr. after it had been set up.

Time has been counted as from the time of application of the dust preparation to the insects.

The rate of respiration of each group of insects was measured at various intervals by measuring their total oxygen uptake over relatively short time intervals. Except overnight, when longer intervals had to be used, the oxygen uptake over 1–4 hr. was measured, the time interval used depending largely upon the rate of oxygen consumption. In this fashion it was possible to obtain a continuous picture of the variations of rate of respiration of groups of insects with time.

Experimental results

The experimental results have been given in graphical form (Figs. 2–14).

Except for Fig. 10, each graph shows the results of one experiment in which a number of groups of insects, taken from the same culture, were treated with various insecticide preparations at approximately the same time.

A curve has been fitted, as described below, to each set of points representing the oxygen uptake of a group of insects. Local irregularities may be accounted for by variable activity of the test subjects. However, in the early stages of a number of experiments, the experimental points fall well below the curves fitted by formulae. This occurs not only for insects treated with poisons, but also in control insects, and may be due to the quiescence of the test subjects resulting from treatment with a dust. It seems unlikely that this phenomenon is due to lack of thermal equilibrium

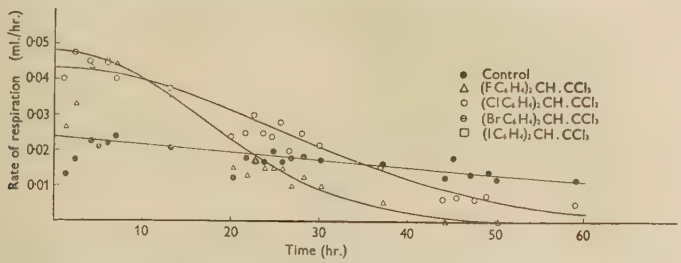

Fig. 2. (Exp.: 26 Nov. 1946.) Effect of halogen analogues of D.D.T. on the respiration of twenty *O. surinamensis*.

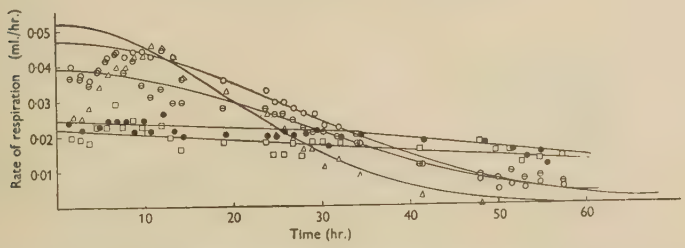

Fig. 3. (Exp.: 2 Dec. 1946.) Effect of halogen analogues of D.D.T. on the respiration of twenty *O. surinamensis*. (Symbols as fig. 2.)

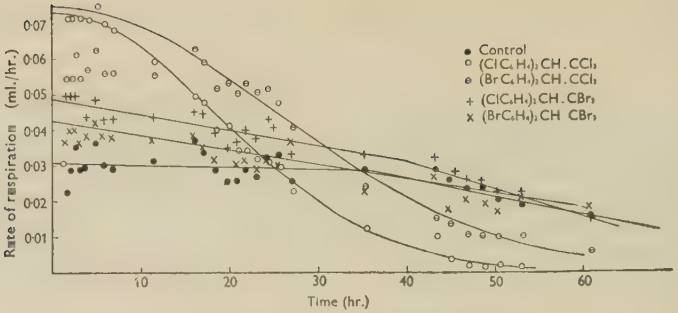

Fig. 4. (Exp.: 9 Dec. 1946.) Effect of halogen analogues of D.D.T. on the respiration of thirty *O. surinamensis*.

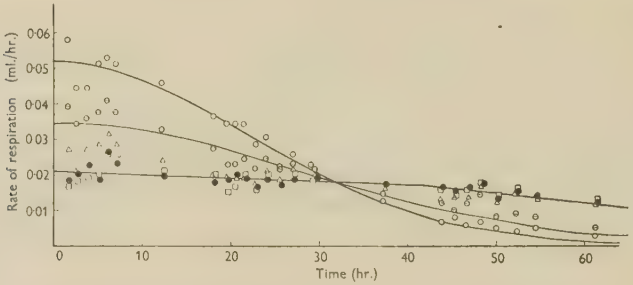

Fig. 5. (Exp.: 16 Dec. 1946.) Effect of D.D.T. analogues on the respiration of twenty *O. surinamensis*. (Symbols as fig. 6.)

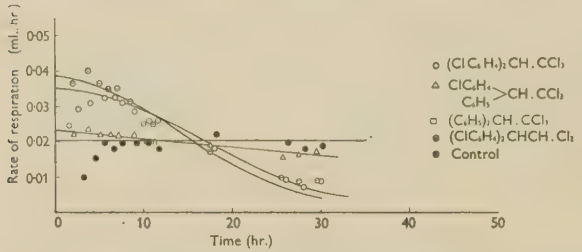

Fig. 6. (Exp.: 14 Jan. 1947.) Effect of D.D.T. analogues on the respiration of twenty *O. surinamensis*.

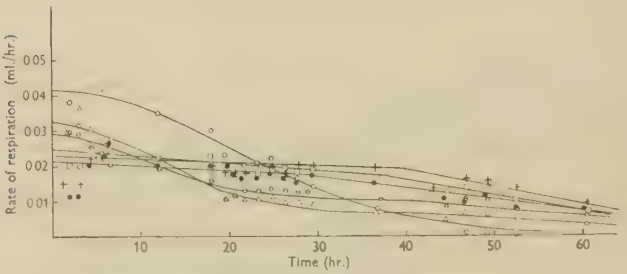

Fig. 7. (Exp.: 30 Dec. 1946.) Effect of alkyloxy analogues of D.D.T. on the respiration of twenty *O. surinamensis*. (Symbols as fig. 8.)

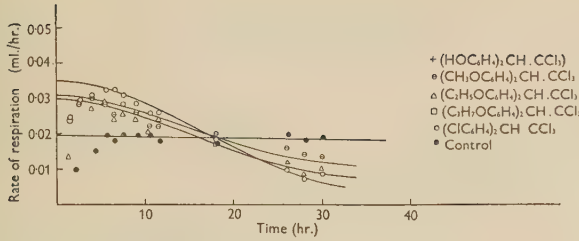

Fig. 8. (Exp.: 14 Jan. 1947.) Effect of alkyloxy analogues of D.D.T. on the respiration of twenty *O. surinamensis*.

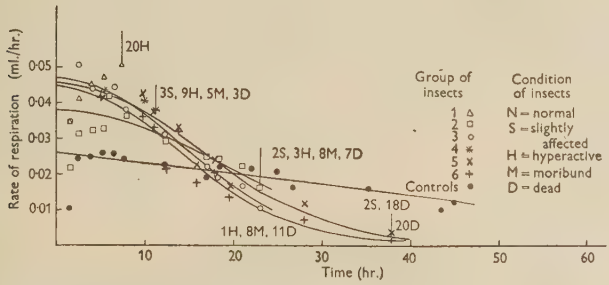

Fig. 9. (Exp.: 6 Jan. 1947.) Correlation of condition of *O. surinamensis* with rate of respiration. (Groups of twenty D.D.T. poisoned insects.)

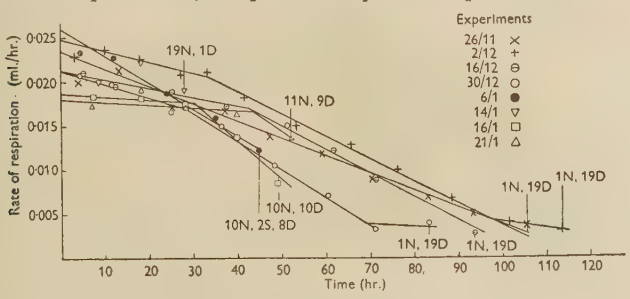

Fig. 10. Correlation of condition of *O. surinamensis* with rate of respiration—control insects.

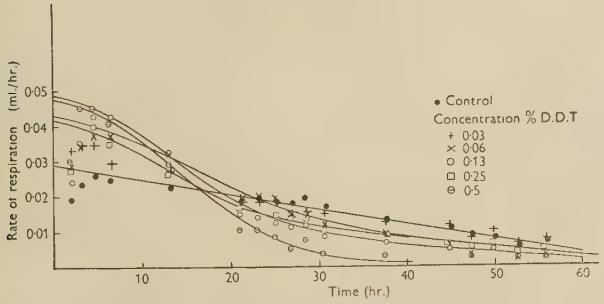

Fig. 11. Exp.: 10 March 1947.) Effect of various concentrations of D.D.T. on the respiration of twenty *O. surinamensis*.

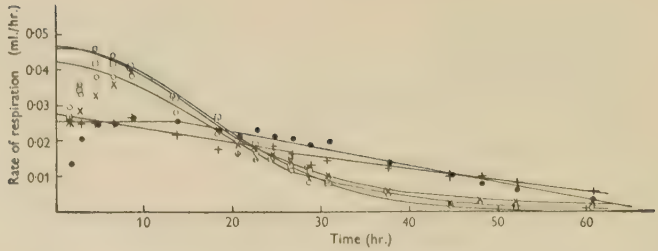

Fig. 12. (Exp.: 17 March 1947.) Effect of various concentrations of D.D.T. on the respiration of twenty *O. surinamensis*. (Symbols as fig. 11.)

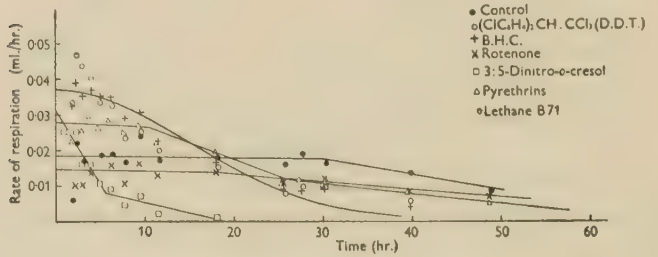

Fig. 13. (Exp.: 16 Jan. 1947.) Effect of various insecticides on the respiration of twenty *O. surinamensis*.

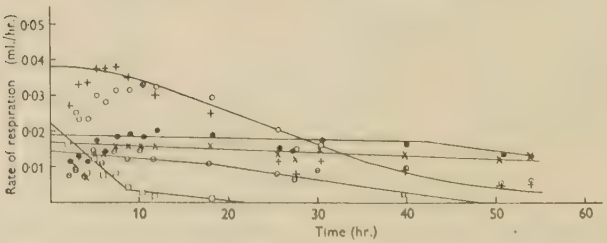

Fig. 14. (Exp.: 21 Jan. 1947.) Effect of various insecticides on the respiration of twenty *O. surinamensis*. (Symbols as fig. 13.)

in the apparatus since the first experimental values are never above the fitted curve and the phenomenon was not observed in all the experiments. Since this discrepancy has been observed both for control and poisoned insects it would not appear to be due to a retarded appearance of toxic symptoms, although it is to be expected that these take a finite time to appear.

No account of the possible delay in the appearance of symptoms of poisoning has been taken in the curves which have been fitted to the experimental data, since the indications are that any rise in rate of oxygen uptake occurred before it was possible to make observations under the experimental conditions described here. The initial 'acceleration' of the rate of respiration is, however, of both practical and theoretical

importance and it is intended to modify the present experimental procedure in order to observe it.*

In Table 1 an attempt has been made to describe the effect of a number of D.D.T. analogues on the respiration of *O. surinamensis* in terms of a number of parameters. The reasons for choosing them are described later in the text.

Rate of respiration-time curves

Curves were fitted to the experimental data in order to systematize and assist in its interpretation. The curves used, which were fitted by eye, were chosen on the grounds of expedience and not for theoretical reasons.

The plot of variation of the rate of oxygen uptake with the time after treatments, obtained when a group of *O. surinamensis* were treated with a stockalite dust containing 5% of any of the following substances (see Figs. 2–6),

$$(\mathrm{F\,C_6H_4})_2\,\mathrm{CH.CCl_3},$$
$$(\mathrm{ClC_6H_4})_2\,\mathrm{CH.CCl_3},$$
$$(\mathrm{BrC_6H_4})_2\,\mathrm{CH.CCl_3},$$
$$(\mathrm{ClC_6H_4})_2\,\mathrm{CH.CHCl_2},$$

was fitted by a curve of the form

$$R = \frac{K}{\sqrt{(2\pi)}}\, e^{-t^2/2b^2}, \qquad (1)$$

where R = rate of respiration t hr. after treatment; K and b are constants.

Although this equation does not give a unique fit to the data, it is very convenient since its values are tabulated (Fisher & Yates, 1938, Table II) and the total area enclosed beneath it (which represents the total oxygen uptake of the insects during their life) is readily determined. Thus it is possible to fit a curve to 'incomplete' sets of data and extrapolate in order to obtain an estimate of the total amount of respiration before death of test subjects on which observations had ceased before respiration had completely stopped.

This curve, however, could not be reasonably fitted to the plot of rate of oxygen uptake R against time t obtained from observations on control insects (Fig. 10) and certain of the less toxic substances. The general shape observed for these substances can be represented by straight lines as shown in Fig. 15*a*.

Sufficient data are not available to assert the existence of the dotted portion of the curve, but experimental results suggest that such an extension is likely. It seems unlikely, however, that straight lines actually represent the data, but when they give a reasonable fit they form a more convenient summary than a more complicated curve.

These data may also be fitted by a sigmoid curve of the form

$$R = K_1 - K_2 \int e^{-K_3 t^2}\, dt, \qquad (2)$$

* This has since been confirmed by a modified technique, the maximum rate of oxygen uptake being attained within 90 min.

TABLE 1. *Respiration of groups of* O. surinamensis. *Effect of D.D.T. and its analogues*

Poison	Molecular weight	Total oxygen uptake ml. (Calculated)	Total oxygen uptake ml. (Experimental at $2b$ hr.)	Index of length of life $\equiv 2b$ hr.	Initial rate of oxygen uptake R_0 ml./hr.	Index of speed of action $r/2b$ (ml./hr.)
Experiment 26/11:						
Control	—	1·42	1·38	119	0·0234	0·0119
$(F\,C_6H_4)_2\,CH.CCl_3$	322	1·00	0·86	33	0·0482	0·0303
$(ClC_6H_4)_2\,CH.CCl_3$	355	1·36	1·27	50	0·0433	0·0272
Mean for D.D.T. analogues		1·18 ± 0·25	—	—	—	—
Experiment 2/12:						
Control	—	1·64	1·60	114	0·0248	0·0144
$(F\,C_6H_4)_2\,CH.CCl_3$	322	1·24	0·99	38	0·0519	0·0326
$(ClC_6H_4)_2\,CH.CCl_3$	355	1·53	1·33	52	0·0470	0·0294
$(BrC_6H_4)_2\,CH.CCl_3$	444	1·31	1·16	53·4	0·0391	0·0245
$(I\,C_6H_4)_2\,CH.CCl_3$	538	1·50	1·53	134	0·0224	0·0112
Mean for D.D.T. analogues		1·40 ± 0·14	—	—	—	—
Experiment 9/12:						
Control	—	1·23	1·23	91	0·0203	0·0135
$(ClC_6H_4)_2\,CH.CCl_3$	355	1·13	0·97	37	0·0487	0·0305
$(BrC_6H_4)_2\,CH.CCl_3$	444	1·57	1·24	50	0·0500	0·0314
$(ClC_6H_4)_2\,CH.CBr_3$	488	1·47	1·41	80	0·0323	0·0183
$(BrC_6H_4)_2\,CH.CBr_3$	577	1·46	1·35	102	0·0287	0·0143
Mean for D.D.T. analogues		1·41 ± 0·19		—	—	—
Experiment 16/12:						
Control	—	1·35	1·32	106	0·0212	0·0127
$(ClC_6H_4)_2\,CH.CHCl_2$	320	1·21	1·17	56	0·0346	0·0216
$(ClC_6H_4)_2\,CH.CCl_3$	355	1·42	1·31	43·6	0·0519	0·0326
$ClC_6H_4 \diagdown C_6H_5 \diagup CH.CCl_3$	320	1·77	—	160	0·0283	0·0111
$(C_6H_5)_2\,CH.CCl_3$	285	1·62	—	149	0·0218	0·0109
Mean for D.D.T. analogues		1·51 ± 0·24	—	—	—	—
Experiment 14/1:						
Control	—	—	—	—	—	—
$(ClC_6H_4)_2\,CH.CHCl_2$	320	0·66	0·60	28	0·0379	0·0236
$(ClC_6H_4)_2\,CH.CCl_3$	355	0·70	0·61	32	0·0350	0·0219
*$(CH_3OC_6H_4)_2\,CH.CCl_3$	346	0·72	—	28	0·0412	0·0257
*$(C_2H_5OC_6H_4)_2\,CH.CCl_3$	374	0·61	—	28	0·0349	0·0218
Experiment 30/12:						
Control	—	1·01	0·96	81	0·0213	0·0125
$(ClC_6H_4)_2\,CH.CCl_3$	355	1·05	0·94	40·2	0·0416	0·0261
$(HOC_6H_4)_2\,CH.CCl_3$	317	1·19	1·17	80	0·0215	0·0149
*$(CH_3OC_6H_4)_2\,CH.CCl_3$	346	0·54	—	19·8	0·0434	0·0273
*$(C_2H_5OC_6H_4)_2\,CH.CCl_3$	374	0·60	—	24·0	0·0398	0·0250
$(C_3H_7OC_6H_4)_2\,CH.CCl_3$	402	1·10	1·10	77	0·0247	0·0143
Mean for D.D.T. analogues		0·90 ± 0·30	—	—	—	—
Experiment 6/1:						
Control	—	1·08	—	83	0·0260	0·0130
$(ClC_6H_4)_2\,CH.CCl_3$ (2)	—	0·88	—	37	0·0379	0·0238
(3)	—	0·81	—	27·6	0·0470	0·0219
(5)	—	0·89	0·79	31·0	0·0457	0·0293
(6)	—	0·75	0·66	27·0	0·0445	0·0278
Means for D.D.T.		0·83 ± 0·07	—	30·9 ± 4·6	0·0438 ± 0·0040	0·0257 ± 0·0034

* Calculated for twenty insects all affected.

a portion of which fits very closely to the curve given by equation (1). It therefore appears that all the rate of respiration-time curves could be equally well described by a curve of the general form given by equation (2). The values of the constants required to fit a curve given by equation (2) to a set of data are not easily determined, neither is the area enclosed by it readily calculated, hence for practical purposes the other types of curve have been fitted.

When the concentrations used were insufficient to give a 100% kill the data could not be represented either by a curve of the form given by equation (1) or by straight lines as could the controls (Fig. 15*a*). However, the data could be fitted by the summation of the two curves, one curve representing the respiration of the insects which were apparently unaffected and behaved as did the control insects, the other curve of the form given by equation (1) representing the respiration of the affected insects. In this way a curve could be obtained which fitted the experimental results (see Fig. 15*a*, *b*, *c*).

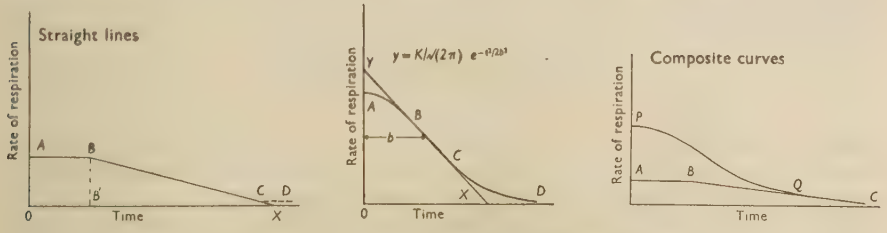

Fig. 15*a–c*. Curves fitted to experimental data.

It was necessary to fit curves of this nature to the data obtained by using the two substances $(CH_3OC_6H_4)_2\,CH.CCl_3$ and $(C_2H_5OC_6H_4)_2\,CH.CCl_3$ (Figs. 7 and 8), and also when various critical concentrations of D.D.T. were used (Figs. 11 and 12).

Experimental fitting of curves

The first step in the fitting of curves to the experimental data was to plot the rate of oxygen uptake of the insects (in ml./hr.) against the time (in hr.) after they were treated with poison.*

Data fitted by curves of the form $R = \dfrac{K}{\sqrt{(2\pi)}}\, e^{-t^2/2b^2}$

The general form of the data to which a curve of the form given by equation (1) can be fitted is that of the curve *ABCD* (Fig. 15*b*). In order to fit the curve, a straight line meeting the axes in *X* and *Y* was drawn by eye through the experimental points where the curve *BC* is almost linear. This line is a tangent to the almost linear part of the curve *BC*.

Then

$$OY = \frac{2Ke^{-\frac{1}{2}}}{\sqrt{(2\pi)}} = 0{\cdot}484K \text{ and } OX = 2b,$$

were measured off to determine the constants K and b, necessary to fix the curve to fit the particular set of data.

* An alternative procedure would have been to plot the logarithm of rate of oxygen uptake against the logarithm of time, in which case the relationship becomes linear. This was not adopted, as such a procedure obscures the relationship between biological effect and respiration rate.

Hence, using Table 2 (Fisher & Yates, 1938), the curve may be constructed

$[t = bx$ and $R = k$ (entry in table corresponding to $x)]$.

On this basis the rate of respiration on application of poison $R_o = 0{\cdot}3989\ K$, and the total respired by the insects before death $r = 1{\cdot}253\ R_o b$ represented by the total area enclosed beneath the curve. $2b$ has been used as a measure of the length of life of the group of insects.

Data fitted by straight lines

Apart from the data obtained using the substance $(CH_3OC_6H_4)_2\,CH.CCl_3$ and $(C_2H_5OC_6H_4)_2\,CH.CCl_3$ and concentrations of D.D.T. not giving 100% kill, data not fitted by curves of the form given by equation (1) were fitted by straight lines. The general shape is shown in Fig. 15*a* although in some cases part *AB* of the curve was not apparent from the observations made.

Straight lines were used to represent as far as possible the data and were fitted by eye. The initial rate of respiration R_o was read off from the point where the straight line *AB* cuts the axis. The time x where *BC* cuts the axis was also determined, and since it may be considered equivalent to a similar quantity estimated in fitting curves of the form given by equation (1) it was designated $2b$ to correspond with this.

The total respired by the insects r was estimated by calculating the area enclosed by $OABX$.

$$r = \tfrac{1}{2}\,(OA + BB')\,OB' + \tfrac{1}{2}\,(BB')\,(B'X).$$

Composite curves

Data to which it is not possible to fit curves in either of the above ways may be fitted by the sum of two curves. The one curve representing the respiration of x normal insects, and the other, y poisoned insects (see above). This is essentially the quantal response upon which probit analysis is based. The curve *ABC* (Fig. 15*c*) represents the respiration of x normal insects and the remainder beneath *PQC* that of y poisoned insects, and the sum of the two *PQC* represents the total respiration of the group. In order to fit the curve it is necessary to estimate x and y, since if x is known *ABC* may be calculated from observations made on the control insects. An estimate of the number of normal insects x may be obtained from the part of the curve *QC*, since at the point *Q* the respiration of the poisoned insects became negligible. Therefore using the part of the curve *QC* and the rate of respiration of the controls we have

$$\frac{x}{\text{Number of controls}} = \frac{\text{Rate of respiration of treated group at time } t^*}{\text{Rate of respiration of control group at time } t}.$$

Hence the respiration of the x unaffected insects can be obtained, and by subtraction the respiration of the y affected insects. It then becomes possible to fit a curve of the form given by equation (1) to the data on the rate of respiration of the y affected insects. It was possible to take this course because of the relationship between the condition of insects and their respiration demonstrated below.

CORRELATION BETWEEN CONDITION OF INSECTS AND RESPIRATION

The rate of oxygen uptake and hence the shape of the respiration curves will depend upon two factors:

(*a*) The decrease of the rate of respiration of individual insects with time.

(*b*) The decrease in the number of survivors with time.

* Time t must be greater than tq at which the respiration of the poisoned insects becomes negligible.

By comparing the rates of respiration of groups of insects with the number of insects surviving after a given time it appears that the respiration of the individual insects remains almost constant until death approaches when the rate of respiration decreases rapidly to a quantity too small to be measured, with the result that the rate of respiration of a group of insects at any time is dependent mainly on the number of surviving insects.

Control insects

At the end of each respiration experiment all the insects were inspected visually using a warm plate (Tattersfield & Potter, 1943) and were classified according to their condition. The insects were classified as *N* normal, *S* slightly affected (obviously not normal but able to walk), *M* moribund and *D* dead. Comparison of the data obtained for the controls in this manner with the corresponding position on the rate of respiration-time curve suggests a rough correlation between rate of respiration and number of insects alive (see Fig. 10). If this relationship actually exists then it is to be expected that the number of surviving insects at any time t after the beginning of the experiment can be approximately determined, and assuming that the respiration of an insect ceases suddenly as it dies, the number N_t of insects surviving at time t from a group containing N live insects at time $t=0$ should be given by the expression

$$N_t = \frac{R_t}{R_0} N_0, \qquad (3)$$

where R_t = rate of respiration of group at time t, R_0 = rate of respiration of group at time $t=0$.

TABLE 2. *Relationship between insects surviving and rate of respiration of groups of* O. surinamensis

	Rate of oxygen uptake ml./hr.			Condition of insects						
Controls: Experiment	Initial R_0	After t hr. R_t	R_t/R_0 N_0*	After t hr. N	S	H†	M	D	'Survivals' $N+S$	
26/11	0·0234	0·0037	3·1	—	1	—	—	19	1	
2/12	0·0248	0·0030	2·4	—	1	—	—	19	1	
9/12	0·0307	0·0027	2·6	—	2	—	—	28	2	
16/12	0·0212	0·0028	2·6	—	1	—	—	19	1	
30/12	0·0213	0·0040	3·8	—	1	—	—	19	1	
14/1	0·0210	0·0195	19	19	—	—	—	1	19	
16/1	0·0186	0·0086	9·2	10	—	—	—	10	10	
21/1	0·0180	0·0135	15	11	—	—	—	9	11	
6/1	0·0260	0·0122	9·4	10	2	—	—	8	12	
D.D.T. treated									$S+H$	$S+H$ $+\frac{1}{2}M$
6/1 (1)	0·0453	0·0474	21	—	—	20	—	—	20	20
(2)	0·0379	0·0162	8·5	—	2	3	8	7	5	9
(3)	0·0470	0·0103	4·3	—	—	1	8	11	1	5
(1*a*)	0·0436	0·0364	17	—	3	9	5	3	12	14·5
(5)	0·0457	0·0034	1·5	—	2	—	—	18	2	2
(6)	0·0445	0·0018	0·8	—	—	—	—	20	0	0

* N_0 = number of insects alive initially.

† H = a new classification for insects. Insects which are unable to progress, but which are in violent movement.

The results set out in Table 2 show that a correlation between $\frac{R_t}{R_0} N_0$ and the number of insects surviving at the end of an experiment exists. The number of insects surviving as estimated from the value of $R_t N_0/R_0$ appears to have a positive bias of one. The standard error of the determination is approximately two. This implies that the rate of respiration falls off suddenly (compared with the rate of decline of the live population).

D.D.T.-poisoned insects

A number of groups of insects were treated with D.D.T. dust and were examined visually at various times after poisoning (Fig. 11). The respiration of each group of insects was observed after which the visual examination of the group was made in the way previously described for use with the controls. It was necessary, however, to introduce a new category H hyperactive, to describe the condition of those insects which were not able to progress, but which were in a state of violent agitation.

The relationship between the number of active insects (S and H) and the rate of oxygen uptake of a group of insects, all suffering from D.D.T. poisoning, can be seen to be similar to that between surviving insects and respiration as observed for control insects (Figs. 10 and 11). D.D.T. stimulates the respiration of insects and if the rate of oxygen uptake remains steady at the high level until the insect becomes moribund, when it suddenly falls to a small value, it is possible to calculate the number of active, as opposed to moribund and dead insects, in a group all suffering from D.D.T. poisoning, by means of the expression:

$$N_t = \frac{R_t}{R_0} N_0, \tag{3a}$$

where R_t = rate of respiration at time t,

R_0 = rate of respiration at time $t = 0$,

N_t = number of active insects at time t,

N_0 = number of insects in group (all D.D.T. poisoned).

The results set out in Table 2 show an approximate agreement between the number of insects surviving and the value $R_t N_0/R_0$ which appears in general to give an overestimate of the highly active insects in the group. This is apparently due to the respiration of the moribund insects which have been classified as dead. A closer relationship is obtained if half the number of insects classified as M are included with insects classified as S and N. Sufficient data to justify this step are not available.

Under these circumstances the number of 'active' insects surviving as estimated by the value $R_t N_0/R_0$ appears to have a positive bias of one and a standard error of approximately two.

Relation between survival curves and respiration curves

If the expressions (3) and (3*a*) are strictly true, then the number of survivors may be deduced directly from the respiration curves, i.e. if

$$N_t = N_0 \frac{R_t}{R_0},$$

and if equation (2) represents the actual decay of the rate of respiration of a group of insects with time, i.e. if

$$R_t = K_1 - K_2 \int_0^t e^{-K_3 t^2}\, dt,$$

then the survival curve will be given by the expression

$$N_t = N_0 \frac{R_t}{R_0} = \frac{N_0}{R_0}\left(K_1 - K_2 \int_0^t e^{-K_3 t^2}\, dt\right).$$

This is the type of expression that would be expected if the length of life of the insects were normally distributed about the mean length of life, viz. if numbers of insects with length of life $t = n = K_a e^{-K_b t^2}$ and number of insects surviving after time $t = N_0 - n = N_0 - \int_0^t K_a e^{-K_b t^2}$. Thus the curve obtained from the respiration experiments may be represented as a survival curve, not only in the case of the controls which die of starvation, but also in the case of the poisoned batches of insects. Pearl & Parker (1924) and also Greiff (1940), working on the length of life of *Drosophila melanogaster* under conditions of starvation, have shown a similar kind of survival curve.

Effect of concentration of D.D.T. in dust preparations

The experiments were carried out at only one level of concentration of poison and it was therefore essential to know the effect of concentration of poison in the dust before attempting to compare the speeds of action of the various poisons.

A preliminary experiment was therefore carried out to determine the 'critical' range of concentrations of D.D.T. in the stockalite carrier, against the test subject *Oryzaephilus surinamensis*. A series of concentrations was prepared by diluting the 5 % dust, as used for comparative purposes, with suitable amounts of the stockalite carrier. The insects were dusted in 2 × 1 in. specimen tubes closed with bolting silk and were kept in contact with the poison powder and kept under the conditions in which they were reared until the inspection was made. They were inspected visually using a warm plate 21 hr. after treatment with dust. Tests at each concentration were carried out in duplicate.

Percentage D.D.T. concentration	$\frac{B+M+D}{\text{No. of insects}}$	Percentage mortality
1·00	43/43	100
0·50	37/37	100
0·25	40/40	100
0·13	18/37	47
0·06	14/38	37
0·03	4/37	11
Control	10/39	26

On the basis of these results the following series of concentrations of D.D.T., 0·03, 0·06, 0·13, 0·25 and 0·5%, were selected for comparison in the respirometers. Two runs were made at each concentration of D.D.T. The results of these experiments are shown in Figs. 11 and 12 and Table 3.

TABLE 3. *Effect of concentration of D.D.T. in dusts on the respiration of groups of twenty* O. surinamensis

Percentage D.D.T. concentration	Estimated percentage kill	Total oxygen uptake* calculated for twenty poisoned insects r ml.	Index of length of life $2b$ hr.	Initial rate of oxygen uptake R_0 ml./hr.	Index of speed of action $r/2b$ ml./hr.
Experiment 10/3:					
Control	0	0·99	68	0·0291	(0·0146)
0·03	5	—	—	—	—
0·06	35	1·26	29·4	0·0683	0·0429
0·13	50	1·06	25	0·0678	0·0424
0·25	35†	0·94	23·2	0·0649	0·0405
0·50	100	0·78	26·6	0·0470	0·0293
Experiment 17/3:					
Control	0	1·05	67·2	0·0254	(0·0156)
0·03‡	0	0·90	80·0	0·0280	(0·0113)
0·06	60	1·13	28·6	0·0515	0·0395
0·13	70	0·91	29·6	0·0489	0·0307
0·25	100	0·96	33·0	0·0466	0·0291
0·50	100	1·03	33·2	0·0458	0·0310

* Where the D.D.T. concentration has given a kill between 0–100% the figures given in these columns are calculated to refer to twenty D.D.T.-poisoned insects. (The unaffected insects are not taken into account in these cases.)

† This result appears exceptional.

‡ Treated as a control.

At the lower concentrations not all of the insects in the groups were affected by the poison; therefore, the number of unaffected insects in each case was estimated from the experimental respiration curves and hence the respiration of the affected insects was determined by fitting composite curves as described above. The value of initial rate of respiration R_0, total oxygen uptake r, $2b$ (determined from the graphs as previously described) an index of the length of life, and rate of toxic action $r/2b$ (see below) for the affected animals, adjusted to apply to groups of twenty affected insects are set out in Table 3. Variations in the total respired by groups of twenty poisoned insects show no systematic effect of concentration of D.D.T. in the poison dust. Again increasing concentration does not affect the length of life ($2b$) of the groups of insects and as may be reasonably expected from this the initial rate of respiration R_0 appears to be unaffected by the concentration of D.D.T. The speed of toxic action as given by $r/2b$ (see below) shows no regular variation with the concentration of poison applied. The two large differences in $r/2b$ which occur, one in each experiment, may be ascribed to chance variations, since no regular trend is to be observed.

The results obtained using 5% D.D.T. dusts on other dates are in accord with the results described above. The results therefore indicate that when an insect is poisoned by D.D.T. the rate of oxygen uptake, total oxygen consumed, and the length of life is not affected by the concentration. This only applies if the concentration is lethal and will not necessarily be true if the D.D.T. is applied in a form other than a dust. Since the analogues of D.D.T. appear to act in a similar fashion to D.D.T. it seems reasonable to extend these conclusions to them.

Respiration of insects treated or untreated with D.D.T. or its analogues

Total oxygen uptake

The total oxygen uptake of control groups of insects and groups treated with D.D.T. or its analogues are set out in Table 1 and are summarized in Table 1*a* (abstracted from Table 1).

Table 1*a*. *Total volumes respired (ml. oxygen) by groups of twenty* O. surinamensis *during life*

Experiment	Control (carrier only)	D.D.T. poisoned (5% dust)	Mean values for all groups tested with D.D.T. and its analogues (5% dusts)
26/11	1·42	1·36	1·18 ± 0·25
2/12	1·64	1·53	1·40 ± 0·14
9/12	1·23	1·13	1·41 ± 0·19
16/12	1·35	1·42	1·51 ± 0·24
30/12	1·01	1·05	0·90 ± 0·30
6/1	1·08	0·83* ± 0·07	0·83 ± 0·07
16/1	0·90	0·61	—
21/1	1·17	1·09	—
Mean values...	1·23 ± 0·26	1·13 ± 0·31	1·21 ± 0·30

* This value is a mean of four results.

The differences which occur between the total oxygen uptake of the control groups and D.D.T.-treated groups are not significantly different as is shown by the standard errors of the mean values.† The mean value of the volume respired by the control groups is 1·23 ± 0·26 and for the D.D.T.-treated groups 1·13 ± 0·31. The mean value of the total respired by groups treated with D.D.T. or one of its analogues is 1·21 ± 0·30, which again is not significantly different from the mean value for the controls.†

It may therefore be concluded that treatment of *O. surinamensis* with D.D.T. or its analogues does not affect the total respired under starvation conditions. This is

† From Table 1 it can be seen that the difference between the maximum, i.e. 'initial' rates of oxygen uptake of groups of twenty normal and twenty D.D.T.-poisoned insects is less than 0·03 ml./hr. Thus the maximum possible correction for the value of the total oxygen uptake of D.D.T.-treated insects is less than 0·03 × 1·5 = 0·045 ml. (see footnote to p. 123), a result so small as not to affect the lack of significant difference in the total oxygen uptakes or the deductions drawn from the data.

not necessarily true of other insecticides having a different mode of action, and it has been shown not to apply to rotenone and Lethane B 71 which definitely reduce the total oxygen uptake.

Since the total volumes respired by similar groups of insects is the same whether or not they are treated with the poison (D.D.T. or its analogues) it may be concluded that the insects die either when their energy reserves, as measured by the amount they respire, are exhausted or when some similar factor associated with death by starvation and depletion of body reserves becomes operative.

TABLE 4. *Correlation between length of life and total oxygen uptake for groups of twenty* O. surinamensis

	Control groups		D.D.T.-treated groups	
Experiment	Total respired *r* ml.	Index of length of life 2*b* hr.	Total respired *r* ml.	Index of length of life 2*b* hr.
26/11	1·42	119	1·36	50
2/12	1·62	114	1·53	52
9/12	1·23	91	1·13	37
16/12	1·35	106	1·42	43·6
30/12	1·01	81	1·05	40·2
14/1	—	—	0·70	32
16/1	0·90	71	0·61	29·6
21/1	1·17	92	1·09	46
6/1	1·08	83	0·88	37
			0·81	27·6
			0·89	31
			0·75	27
10/3	0·99	68	—	—
	1·05	67·2	—	—
Mean values...	1·18	89·2	1·02	37·8

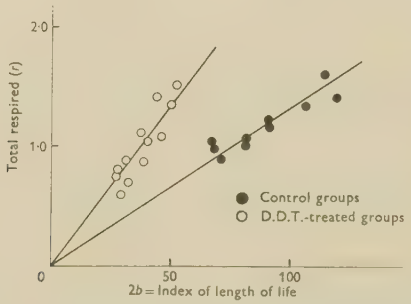

Fig. 16. Relation between length of life and total respired.

Relation between total oxygen uptake and length of life

The relationship between *r*, the total volume respired by a group of insects, and 2*b*, a measure of the length of life of the members of the group, is linear, provided that each group contains the same number of insects and is similarly treated (Table 4)

as may be shown from the results set out in Table 4 (see Fig. 16), so that the general relationship may be expressed by the equation

$$r = 2\mathbf{K}b = C,$$

where $\mathbf{K}$ = constant for each treatment and may be calculated for the controls and D.D.T.-treated insects from Table 4, $C = 0$ in our case, since for a group of insects where the 'length of life' $2b = 0$, $r = 0$. Thus the relationship between r and b for the controls may be represented by

$$r/2b = \mathbf{K} = 0{\cdot}0132 \pm 0{\cdot}0017,$$

and for D.D.T.-treated insects by

$$r/2b = \mathbf{K} = 0{\cdot}0272 \pm 0{\cdot}0048.$$

Method of comparison of speeds of toxic action

Since it has been shown that $r = 2\mathbf{K}b$, both for control groups of insects and groups poisoned with D.D.T., a method of comparing the speed of action of D.D.T. and its analogues may be devised as below:

If in any population of insects b_n is a measure of the length of life of normal insects under starvation conditions and b_p is a measure of the length of life of poisoned insects, the total respired by the untreated group $= r_n = 2\mathbf{K}_n b_n$ and the total respired by the treated group $= r_p = 2\mathbf{K}_p b_p$, where $\mathbf{K}_n$ and $\mathbf{K}_p$ are the constants for untreated and treated insects respectively.

Now, since the total amounts respired are the same in both cases if the groups are similar,

$$r_n = r_p = 2\mathbf{K}_n b_n = 2\mathbf{K}_p b_p,$$

and hence

$$\mathbf{K}_p = \mathbf{K}_n \frac{2b_n}{2b_p} = r/2b_p,$$

but b_n/b_p is a measure of the speed of action of the poison; hence $\mathbf{K}_p$ is also a measure of the speed of action of the poison. Thus the relative speeds of toxic action of these poisons may best be derived from the values of $\mathbf{K}_p = r_p/2b_p$, since this method will reduce the effect of chance variations between groups of insects selected from the same population, due to different energy reserves in individual insects.

Relative speeds of action of D.D.T. and its analogues

Comparison of the values of $2b$ (Table 1; Fig. 17*b*), which is a measure of the length of life of a group of insects, shows a general trend in the direction of a shorter 'life' for insects which have been treated with a poison of lower molecular weight, i.e. the speed of toxic action and molecular weight are negatively correlated. This is confirmed by the values of $r/2b$ (Table 5; Fig. 17*d*) which probably give a better estimate of the speed of toxic action than the values of $\frac{1}{2}b$ (see above). Since the *total* volume of oxygen respired by a group of insects is apparently unaffected by treatment with these substances the *initial* rate of respiration should also show

a negative correlation with molecular weight. This has actually been observed and a greater stimulation of respiration appears to be caused by poisons of lower molecular weight (Table 1; Fig. 17*a*).

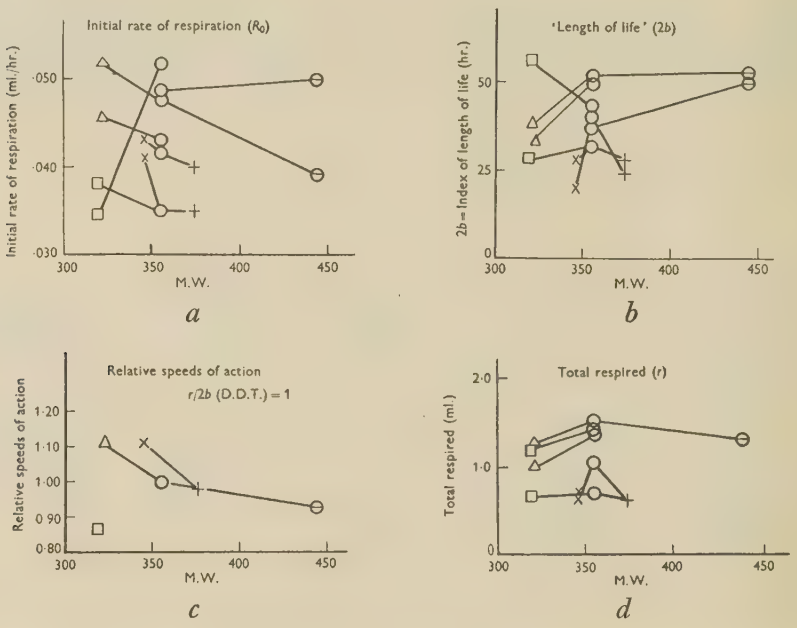

Fig. 17*a–d*. Relation between molecular weight and action of D.D.T. analogues on *O. surinamensis*.

△ $(FC_6H_4)_2CH.CCl_3$ ⊖ $(BrC_6H_4)_2CH.CCl_3$ × $(CH_3OC_6H_4)_2CH.CCl_3$
○ $(ClC_6H_4)_2CH.CCl_3$ □ $(ClC_6H_4)_2CH.CHCl_2$ + $(C_2H_5OC_6H_4)_2CH.OCl_3$

These results do not appear to hold consistently for 1, 1-*bis* (*p*-chlorophenyl) 2, 2-dichloroethane which is not, however, a member of the same type of analogous series as the other substances.

TABLE 5. *Relative speeds of action*

Comparison of index of speeds of action *r*/2*b*.

Substance				Mean value
$(FC_6H_4)_2CH.CCl_3$	0·0303 (1·11)	0·0326 (1·11)	—	(1·11)
$(ClC_6H_4)_2CH.CCl_3$	0·0272 (1·00)	0·0294 (1·00)	0·0305 (1·00)	(1·00)
$(BrC_6H_4)_2CH.CCl_3$	—	0·0245 (0·83)	0·0314 (1·03)	(0·93)
$(ClC_6H_4)_2CH.CHCl_2$	0·0216 (0·66)	0·0236 (1·08)	—	(0·87)
$(ClC_6H_4)_2CH.CCl_3$	0·0326 (1·00)	0·0219 (1·00)	0·0261 (1·00)	(1·00)
$(CH_3OC_6H_4)_2CH.CCl_3$	—	0·0257 (1·17)	0·0273 (1·05)	(1·11)
$(C_2H_5OC_6H_4)_2CH.CCl_3$	—	0·0218 (1·00)	0·0250 (0·96)	(0·98)

The figures given in brackets are adjusted so that *r*/2*b* for D.D.T. = 1·00 in all cases.

The relatively long time required for all members of a group of insects to die after individuals in the group have begun to die (see 'Correlation between condition of insects and rate of respiration', p. 126) is important, because the length of life varies more from individual insect to insect than it does from analogue to analogue. This must be taken into account when the speeds of toxic action of various poisons are compared, since it is likely to cause large discrepancies in individual determinations of speed of toxic action, when small numbers of insects are used. The magnitude of variation to be expected is given by the standard errors determined in the experiment 16/1 when a number of parallel determinations were carried out using D.D.T.; the value of $r/2b$ was $0{\cdot}0257 \pm 0{\cdot}0034$ (see Table 1). In general the differences in speed of action (Table 3) are of about this order, and in the absence of a general trend with molecular weight could probably be dismissed as being within experimental error. Nevertheless, it appears that there is a tendency for the toxic action of D.D.T. analogues towards *O. surinamensis* to be more rapid the lighter the molecules, although differences in speed of action are small.

Discussion of results

Speed of action

It has been reported that in Germany the fluorine analogue of D.D.T. has been used in preference to D.D.T. (Kilgore, 1945) because its speed of action has made it more useful for certain purposes than the more potent and cheaper product D.D.T. At first sight this statement appears to be at variance with the results obtained during the course of the experiments described here which indicate that there is only a very slight difference in speed of toxic action between D.D.T. and its analogues. However, the results are not contradictory since in these experiments it has been the time of actual occurrence of death which has been measured and not the time required for 'knock down' or the rendering of the insects incapable of normal activities, which is probably the time factor linked with efficiency for control purposes, and there is no reason why these two quantities should necessarily be related.

Thus Prill, Hartzell & Arthur (1945) and Prill, Synerholm & Hartzell (1946) have shown that although certain alkyloxy analogues of D.D.T. possess a higher knock down activity than D.D.T. to houseflies, they are not so toxic. These analogues do not appear to kill *O. surinamensis* at a correspondingly quicker rate than D.D.T.

Unfortunately, it was not possible to make observations sufficiently quickly to obtain a clear picture of the behaviour of the respiration of the test subjects for the time immediately after treatment, so that the incidence of 'knock down' to this test subject was not observed.

Results in relation to work on mode of action of D.D.T.

Roeder & Weiant (1946) have concluded that in the cockroach the tremors characteristic of D.D.T. poisoning are due to an intense and patternless bombardment of the motor neurons by trains of impulses originating in sensory endings, as

a result of the action of D.D.T. on unidentified nerve structures. They also conclude that D.D.T. has no significant action on the central nervous system. This, taken in conjunction with the fact that the total amounts respired by insects before death, under starvation conditions, is the same whether they are untreated or are treated with D.D.T. (or one of its analogues), suggests a possible mode of action for D.D.T. Thus when D.D.T. comes into contact with an insect it penetrates to the sensitive part of the nervous system, where it sets up trains of impulses and thereby causes the insect to become hyperactive, with the result that the insect ultimately dies of exhaustion. In the case of *O. surinamensis* the energy reserves appear to become exhausted, since poisoned insects respire to the same extent as untreated insects maintained under starvation conditions. While this is true of *O. surinamensis* it would be unreasonable to assume that this will be the case with all insects, and whilst the depletion of energy reserves is likely to be at least an important contributory cause of death, it is possible that death is actually caused by some other breakdown arising from hyperactivity. Therefore it is suggested that insects treated with D.D.T. do not die as a direct result of D.D.T., but that the life of this insect is shortened as a result of its increased activity, which results from the action of D.D.T. Under normal conditions a D.D.T.-poisoned insect is unable to feed as a result of the hyperactivity induced by the D.D.T., and as a result the insect's energy reserves are depleted. The depletion of energy reserves may or may not be the primary cause of death. This is supported by the observation that caterpillars treated with D.D.T. which begin to eat almost invariably recover from the effects of D.D.T. poisoning although they may show the symptoms of the poisoning (M. J. Way, priv. comm.). Again Merrill, Savit & Tobias (1946) have shown that administration of glucose to D.D.T.-poisoned cockroaches prolongs the life of the insects. Because prevention of carbohydrate depletion by administration of glucose or prolonged anaesthesia does not alter the final course of the poisoning they conclude that these changes are not critical in the cause of death. They do not, however, consider the possibility that the hyperactivity of the insect may be closely associated with the cause of death, and have not presented any evidence which makes this suggestion unlikely.

Alpatov & Pearl (1929), working on the effect of temperature on the longevity of *Drosophila melanogaster*, have concluded that the length of life is dependent upon the rate of expenditure of energy during life. Thus on the evidence put forward above it seems reasonable to suppose that D.D.T. acts by stimulating insects and causing them to 'burn out'. Any alternative theory of mode of action in the face of this evidence must postulate a killing mechanism in addition to the stimulation mechanism. The mechanism put forward here does explain the observed symptoms of D.D.T.-poisoning, viz. an initial stimulation, which takes place relatively rapidly and is followed by a slow death.

Comparison of the effects of D.D.T. and other insecticides on insect respiration

The effects of dusts containing B.H.C., rotenone, Lethane B 71, 3:5-dinitro-*o*-cresol and pyrethrins on the respiration of groups of *Oryzaephilus surinamensis* were observed, using the same technique as in the experiments on the D.D.T. analogues. The results are set out in Figs. 13 and 14. Apart from the pyrethrins the poisons were applied as dusts containing 5% of the toxic agent. The pyrethrins were applied as a powder containing approximately 1% of pyrethrins, prepared from a concentrate rich in pyrethrin I. It was not possible to prepare a free-flowing dust with a higher pyrethrin content owing to the nature of the pyrethrin concentrate available. In all cases stockalite was used as the carrier.

In general it has been possible to make only qualitative comparisons of the actions of the various poisons, and to separate the poisons into two groups. One group stimulates the respiration of *O. surinamensis* and is represented by D.D.T. and its analogues, B.H.C. and the pyrethrins, and a second group depresses the respiration of *O. surinamensis* and is represented by rotenone and Lethane B 71; 3:5 dinitro-*o*-cresol also appears to belong to this latter category.

B.H.C. γ-isomer

The effects of γ-B.H.C. and D.D.T. on the rate of respiration of *O. surinamensis* are almost indistinguishable and the experimental respiration time curves may be represented, in both experiments, by the same line. Thus the final effects of D.D.T. and γ-B.H.C. are apparently similar, and it therefore appears that the mode of action of the two substances is similar.

Pyrethrins

The initial effect of the pyrethrin dust was a stimulation in the rate of respiration of *O. surinamensis* followed by a fall in respiration as the insects died. The relative concentrations of pyrethrin I to pyrethrin II (in the ratio of 10:1) make it likely that the effect observed was due to pyrethrin I rather than to pyrethrin II. In view of the very rapid knock-down effect of the pyrethrins it would be of great interest to carry out further investigations using relatively pure pyrethrin I and pyrethrin II.

Lethane B 71 and rotenone

The effect of both these poisons is to depress the rate of respiration of *O. surinamensis*. Under the conditions of the experiment Lethane kills more rapidly than rotenone. Further work on these substances is necessary, however, to decide whether this is due to concentration effect or whether Lethane is intrinsically quicker in action on *O. surinamensis* than rotenone.

3:5-*Dinitro-o-cresol*

Dinitro-cresol is outstanding among all the poisons tested on account of its high speed of action. In both experiments the rate of respiration of the test groups of insects were reduced almost to zero by dinitro-cresol in 10 hr.

Using a modified technique it has been shown that dinitro-cresol causes a rapid initial rise in the oxygen uptake of *Tribolium castaneum*, the maximum rate being about ten times that of normal insects about 1 hr. after application.

I wish to acknowledge the advice and encouragement which Dr Tattersfield has given throughout the work described here. My thanks are also due to Mr F. J. Anscombe for advice on statistical matters.

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THE TRANSFORMATION OF LOCAL LESION COUNTS FOR STATISTICAL ANALYSIS

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(With 4 Text-figures)

An analysis of the frequency distribution of local lesions, produced by viruses on half-leaves of a number of plants, shows that their standard error increases with increasing mean. Hence analysis of variance and statistical tests of significance should not be applied to lesion numbers unless they are suitably transformed. The transformation into logarithms over-corrects so that the standard error decreases with increasing mean. A satisfactory transformation is $y = \log_{10}(x+c)$, where x is the number of lesions and c is a constant. A method is given of assessing c for different experiments. Great accuracy is not needed; in an experiment discussed in detail a satisfactory transformation is obtained with any value for c between 15 and 80.

On individual plants the numbers of lesions formed on half-leaves are distributed more or less normally, whereas their distribution about the common mean for many plants is skew and 'leptokurtic'. The distribution of the transformed numbers is almost normal, both for individual plants and about the common mean for a number of plants.

The local lesion method of assaying infectivity of plant virus preparations has been extensively used since 1929 when it was originally introduced by Holmes (1929). Youden & Beale (1934) early showed the need for statistical design and for analysis of variance in local lesion experiments, because different plants and also different leaves on the same plant can vary considerably in susceptibility. Since then many workers have analysed their results by currently used statistical methods, some applying them directly to numbers of lesions, whereas others applied them to logarithms of numbers of lesions. The methods are based on the assumption that the variate is normally distributed and that the variance is independent of the mean of the population. In the absence of any study of the distribution of local lesions the most appropriate method of their statistical treatment remained uncertain. The work described in this paper was undertaken to remedy this deficiency.

Some departure from a normal distribution is to be expected for lesion numbers, being integers, are not a continuous variate whereas only a continuous variate can give an exact normal distribution. Nevertheless, because fairly large numbers of lesions are usually involved, the intervals between two possible values can be relatively small and numbers of lesions or some function of them might approximate closely enough to the normal distribution for customary statistical analysis to be applicable.

EXPERIMENTAL

Preliminary experiments were made with tobacco mosaic and tomato bushy stunt viruses using *Nicotiana glutinosa* as a test plant. It was observed that the distribution of numbers of lesions formed on each half-leaf about the common mean for a number of plants was strongly skew, and 'leptokurtic', and very often 'bimodal'. It was also noticed that standard errors obtained for each plant separately tend to increase with the increasing mean number of lesions per half-leaf on the same plant. Similar phenomena were observed with a tobacco necrosis virus inoculated into French bean (*Phaseolus vulgaris*, var. Prince). Beans, having only two inoculable leaves which are of the same age and occupy the same position on the plant, present a simpler problem than *Nicotiana glutinosa* and were chosen for a detailed study. The primary leaves of young bean plants were inoculated by rubbing their surface with the fore-finger wet with the sap expressed from infected tobacco plants diluted in distilled water. After inoculation the leaves were washed by spraying with water. Lesions were counted on each half-leaf 4 days after inoculation, when they were well formed but still discrete. Several experiments were made, but results of only one will be considered here in detail. In this experiment 120 plants (480 half-leaves) were inoculated with the sap diluted 1/100. The results of individual lesion counts are given in Table 1.

DISTRIBUTION OF NUMBERS OF LESIONS

A test for departure from normality of the distribution of numbers of lesions on each half-leaf around the common mean for all plants can be made by finding the values of g_1 and g_2 based on the use of k-statistics introduced by Fisher (1928, 1938). A measure of skewness is given by the magnitude of g_1 and that of 'kurtosis' by the magnitude of g_2. Significance of these values can be assessed by comparing them with the standard deviations for normal samples of the same size. These standard deviations were obtained by Fisher (1931, 1938).

The following values are obtained for the numbers of lesions:

$$k_2 = 3002{\cdot}5, \quad k_3 = 139{,}780 \quad \text{and} \quad k_4 = 7{,}251{,}500.$$

Thus
$$g_1 = \frac{k_3}{k_2^{\frac{3}{2}}} = 0{\cdot}850 \quad \text{and} \quad g_2 = \frac{k_4}{k_2^2} = 0{\cdot}804.$$

Variances of g_1 and g_2 for normal samples based on 480 values are 0·0124 and 0·0495, respectively. Therefore the standard deviation of g_1 is 0·111 and that of g_2 is 0·222. The values of g_1 and g_2 obtained from the data exceed nearly eight times and four times, respectively, the values of these standard deviations, showing that the distribution departs considerably from normal, and is skew and 'lepto-kurtic'. This can also be seen from the distribution diagram given in Fig. 1 A.

For the distribution of the plant totals we get in the same way:

$$k_2 = 39{,}915{\cdot}7 \quad \text{(estimate of variance)},$$
$$k_3 = 5{,}611{,}107 \quad \text{and} \quad k_4 = 733{,}111{,}594.$$

Thus
$$g_1 = 0{\cdot}704 \quad \text{and} \quad g_2 = 0{\cdot}460.$$

TABLE 1. *Numbers of lesions formed on half-leaves of* 120 *plants*

59	67	73	84	76	78	83	78	168	118	41	43	92	64	53	60	95	159	143	120	63	44	126	74
56	65	93	56	79	96	56	106	92	130	33	66	156	123	35	21	129	144	139	178	92	75	101	79
60	88	42	16	78	91	166	125	95	91	70	44	15	25	109	132	79	59	34	49	71	50	50	35
89	62	22	13	88	87	158	185	71	77	66	71	32	36	67	70	73	51	84	44	59	48	69	47
103	98	103	129	159	93	148	120	139	155	115	98	81	91	172	95	40	54	157	198	28	33	30	49
63	80	172	137	109	188	69	47	84	141	108	91	29	70	170	213	50	33	187	160	69	48	78	80
72	90	201	225	159	174	49	68	71	77	152	150	76	80	144	110	100	80	164	182	57	22	122	120
153	73	220	120	166	185	39	44	59	50	203	105	42	50	210	142	67	93	212	234	56	55	200	170
72	32	84	59	32	61	71	65	150	131	105	105	107	71	132	54	42	32	136	120	98	79	153	140
65	59	85	102	84	46	30	53	124	96	146	40	62	96	113	76	65	126	126	138	74	46	195	117
167	154	123	108	140	107	131	175	128	129	134	125	85	88	85	107	203	161	65	85	81	95	155	172
212	224	160	120	123	110	189	131	150	108	143	104	56	76	102	81	184	157	72	46	78	65	130	129
141	177	188	233	109	84	134	250	63	86	97	101	30	35	144	106	60	48	91	130	66	88	71	88
166	150	174	187	65	66	207	294	83	70	108	120	23	26	163	190	118	95	114	193	56	49	131	103
130	139	100	84	163	211	103	117	6	9	83	57	185	135	93	78	72	52	62	60	45	23	145	90
142	130	80	105	169	97	147	110	8	11	102	78	100	154	105	108	107	105	88	57	35	44	189	127
29	10	92	161	160	193	303	250	82	68	112	78	105	67	111	161	103	88	13	8	300	232	58	96
26	43	132	85	104	136	215	130	107	106	76	79	107	50	188	143	95	78	23	25	233	173	79	55
118	87	181	207	84	117	128	105	69	135	35	55	19	10	100	86	64	60	60	37	153	104	245	289
90	106	151	143	78	130	93	96	120	156	60	85	12	9	70	77	83	71	71	53	82	100	286	260

Total 49,118. Mean 102·3.

Each rectangle represents one plant. Two separate leaves are represented by two rows and left and right half-leaves by left and right columns.

For normal samples of 120 values the standard deviations of g_1 and g_2 are 0·2209 and 0·4383, respectively. The distribution is, therefore, skew. This can also be seen from the diagram in Fig. 2.

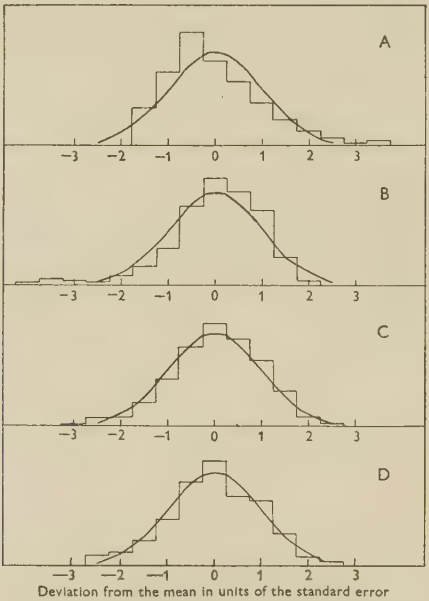

Fig. 1. The histograms of the distributions about their common mean for all plants. A, of numbers of lesions formed on each half-leaf; B, of the function $y = \log x$; C, of the function $y = \log(25+x)$; D, of the function $y = \log(40+x)$ (where x is the number of lesions formed on each half-leaf). The smooth curve is the normal distribution curve with the same variance and the same mean.

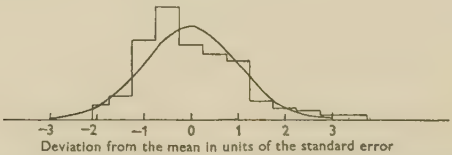

Fig. 2. The histogram of the distribution of total numbers of lesions formed on each plant. The smooth curve is the normal distribution curve with the same variance and the same mean.

From the results of analysis of variance of the numbers of lesions, shown in Table 2, it can be seen that the ratio of the variances between plants and within plants is very high. Variation in numbers of lesions between different plants is, therefore, much greater than that between different half-leaves on the same plant, which is, indeed, obvious from a mere inspection of Table 1. The distribution of the

TABLE 2. *Analysis of variance of numbers of lesions*

	D.F.	Sums of squares	Mean square (variance)	Variance ratio
Between plants	119	1,187,491	9,978·9	14·3
Within plants	360	250,715	696·4	—
Total	479	1,438,206	3,002·5	—

numbers of lesions on each half-leaf about the common mean for all plants will, therefore, be expected to reflect the distribution of the plant totals. The similarity between the diagrams in Figs. 1 A and 2 suggests that it is so. It is, therefore, obvious that if, by application of analysis of variance, variation between the plants could be eliminated, precision in assessing the results would be greatly increased and the fact, that the distribution of the numbers of lesions on each half-leaf about the common mean for all plants differs considerably from normal, would cease to matter. The problem will, however, then be complicated by the fact that instead of one sample of values, we shall have n independent samples with m values in each, each sample drawn from a population with its own mean. Analysis of variance can be applied to such data if distribution of values of each sample around its own population mean is not very different from normal and if all the populations, represented by the samples, have the same variance. This is so because the estimate of the variance will then be

$$s^2 = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m}(x_{ij}-\bar{x}_i)^2}{n(m-1)}$$

(where x_{ij} is the number of lesions on jth half-leaf of ith plant, and $\bar{x}_i$ the mean value of x on ith plant), and the probability differential of s^2 will have the form

$$dp = \left(\frac{v}{2\sigma^2}\right)^{\frac{1}{2}v}\left(\Gamma\frac{v}{2}\right)^{-1} s^{v-2}\, e^{-vs^2/2\sigma^2}\, ds^2 \quad [v=n(m-1)]$$

(on which currently used tests of significance are based), only if within populations, represented by the separate independent samples, the variate is distributed normally, and if σ^2 is the variance in all the populations.

When there are n independent samples with m values in each taken from the same population, or from populations which differ only in that they have different means, a test for deviation from normality can be made by finding the values of G_1 and G_2, which differ from g_1 and g_2 in that they are based on means of k-statistics found for each separate sample.

Thus $$G_1 = \frac{\bar{k}_3}{\bar{k}_2^{\frac{3}{2}}} \quad \text{and} \quad G_2 = \frac{\bar{k}_4}{\bar{k}_2^2}.$$

For normal samples the standard deviations of G_1 and G_2 can be obtained as follows:

Use is made of the fact that distributions of G_1 and G_2 are independent of the distribution of $\bar{k}_2$. This follows from the fact that distributions of g_1 and g_2 are independent of the distribution of k_2 (for the proof of this, see Fisher, 1931).

Therefore $$E(G_1^2\bar{k}_2^3) = E(\bar{k}_3^2) = E(G_1^2)\,E(\bar{k}_2^3)$$

and $$E(G_2^2\bar{k}_2^4) = E(\bar{k}_4^2) = E(G_2^2)\,E(\bar{k}_2^4),$$

where the operator E stands for 'expectation'. It follows that

$$\operatorname{var} G_1 = E(G_1^2) = \frac{E(\bar{k}_3^2)}{E(\bar{k}_2^3)}$$

and

$$\operatorname{var} G_2 = E(G_2^2) = \frac{E(\bar{k}_4^2)}{E(\bar{k}_2^4)}.$$

It is known that for a normal sample of m values

$$\operatorname{var} k_3 = E(k_3^2) = \frac{6m}{(m-1)\,(m-2)}\,\kappa_2^3$$

and

$$\operatorname{var} k_4 = E(k_4^2) = \frac{24m(m+1)}{(m-1)\,(m-2)\,(m-3)}\,\kappa_2^4,$$

where κ_2 stands for the second cumulant of the population. Thus for the mean values of k_3 and k_4 based on n independent samples with m values in each, we have

$$\operatorname{var} \bar{k}_3 = E(\bar{k}_3^2) = \frac{6m}{n(m-1)\,(m-2)}\,\kappa_2^3$$

and

$$\operatorname{var} \bar{k}_4 = E(\bar{k}_4^2) = \frac{24m(m+1)}{n(m-1)\,(m-2)\,(m-3)}\,\kappa_2^4.$$

The moment generating function of $\bar{k}_2$ is

$$\left(1 - \frac{2\kappa_2\alpha}{v}\right)^{-\frac{1}{2}v},$$

where $v = n\,(m-1)$. Therefore

$$E(\bar{k}_2^r) = \frac{d^r}{d\alpha^r}\left(1 - \frac{2\kappa_2\alpha}{v}\right)^{-\frac{1}{2}v} \text{ at } \alpha = 0.$$

Thus

$$E(\bar{k}_2^3) = \frac{[n(m-1)+2]\,[n(m-1)+4]}{n^2(m-1)^2}\,\kappa_2^3,$$

and

$$E(\bar{k}_2^4) = \frac{[n(m-1)+2]\,[n(m-1)+4]\,[n(m-1)+6]}{n^3(m-1)^3}\,\kappa_2^4.$$

Therefore

$$\operatorname{var} G_1 = \frac{E(\bar{k}_3^2)}{E(\bar{k}_2^3)} = \frac{6nm(m-1)}{(m-2)\,[n(m-1)+2]\,[n(m-1)+4]}$$

and $$\operatorname{var} G_2 = \frac{E(\bar{k}_4^2)}{E(\bar{k}_2^4)} = \frac{24n^2m(m+1)\,(m-1)^2}{(m-2)\,(m-3)\,[n(m-1)+2]\,[n(m-1)+4]\,[n(m-1)+6]}.$$

Treating numbers of lesions on four half-leaves of each plant as an independent sample, we get:

$$G_1 = \frac{\bar{k}_3}{\bar{k}_2^{\frac{3}{2}}} = \frac{-2764}{(696{\cdot}4)^{\frac{3}{2}}} = -0{\cdot}152$$

and

$$G_2 = \frac{\bar{k}_4}{\bar{k}_2^2} = \frac{335{,}055}{(696{\cdot}4)^2} = 0{\cdot}691,$$

and standard deviations of G_1 and G_2 for 120 independent normal samples with 4 values in each are 0·181 and 0·803, respectively. Therefore, if all the populations represented by the samples have the same standard deviation, there is no reason to suspect that their distribution appreciably deviates from normal. Even if standard deviations of the populations are different, it can still be concluded that the distribution within each population probably does not differ greatly from normal, provided it is basically the same in all the populations, and there is no reason to believe this to be otherwise, except, perhaps, for plants with very few lesions.

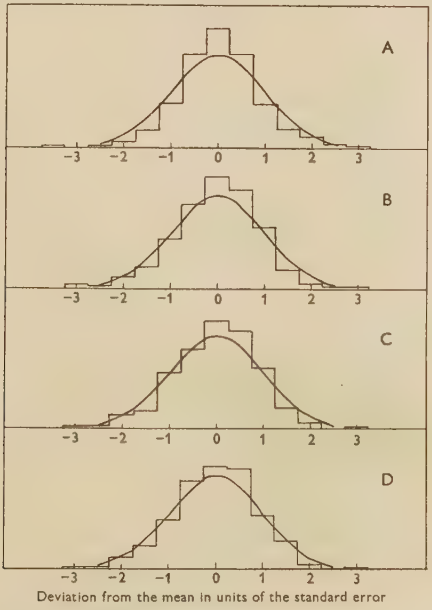

Fig. 3. The histograms of distributions around their means on each plant. A, of numbers of lesions formed on each half-leaf; B, of the function $y = \log x$; C, of the function $y = \log(25+x)$; D, of the function $y = \log(40+x)$ (where x is the number of lesions formed on each half-leaf). The smooth curve is the normal distribution curve with the same variance and the same mean.

If standard errors of the populations were independent of their means, the regression coefficients of the standard error obtained separately for each sample on the mean of the sample would not be expected to differ significantly from zero. The value of the regression coefficient is

$$b = \frac{\Sigma s_i(X_i - \bar{X})}{\Sigma (X_i - \bar{X})^2} = 0{\cdot}161,$$

where s_i is the standard error on a given plant, X_i the mean number of lesions per leaf on the same plant and $\bar{X}$ the mean number of lesions per leaf for all the plants.

It should be pointed out that, if b differs significantly from zero, the precision of separate measurements of s_i will depend on the magnitude of X_i. The above formula will, therefore, give an unweighted estimate of b.

A 'dot diagram' in Fig. 4 A shows the standard error for each plant plotted against the mean numbers of lesions per leaf on the same plant, and the regression line

$$S = \bar{s} + b\,(X - \bar{X}) = 23{\cdot}1 + 0{\cdot}161\,(X - 102{\cdot}3)$$

is also shown. A glance at the figure will show that the standard errors are not independent of the means, but increase with the increasing means.

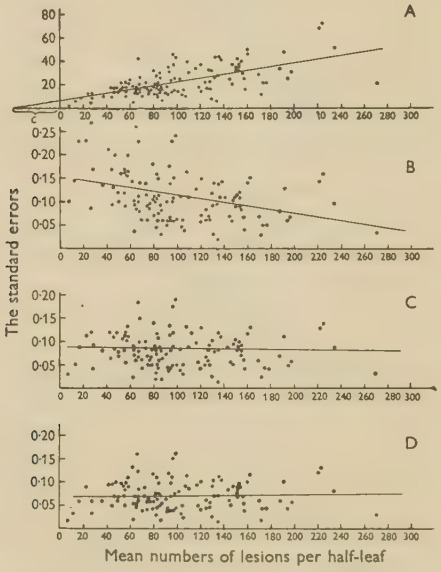

Fig. 4. The 'dot diagrams' of the standard errors for each separate plant. A, of numbers of lesions formed on each half-leaf; B, of the function $y = \log x$; C, of the function $y = \log(25 + x)$; D, of the function $y = \log(40 + x)$ and regression lines of the standard errors on the mean number of lesions per half-leaf on each plant.

The variance of the regression coefficient b is

$$\operatorname{var} b = \frac{\Sigma(\operatorname{var} s_i)(X_i - \bar{X})^2}{[\Sigma(X_i - \bar{X})^2]^2}.$$

If it is assumed that in each population of numbers of lesions, represented by a sample of four values, distribution around its own mean is normal, or, at least, not greatly different from normal (which seems probable from the results of the test for deviation from normality given above), an estimate of the variance of s_i can be obtained, at least approximately, directly from the cumulant generating

function of the standard error based on a normal sample of m values. The function is:

$$\log_e\left\{\left(\frac{m-1}{\sigma_i^2}\right)^{\frac{1}{2}(m-1)} 2^{-\frac{1}{2}(m-3)}\left(\Gamma\frac{m-1}{2}\right)^{-1}\int_0^\infty s^{m-2}\exp\left[-\frac{m-1}{2\sigma_i^2}s^2\right]e^{\alpha s}\,ds\right\}$$

$$=\alpha\left\{\left(\frac{2}{m-1}\right)^{\frac{1}{2}}\left(\Gamma\frac{m}{2}\Big/\Gamma\frac{m-1}{2}\right)\right\}\sigma_i+\frac{\alpha^2}{2}\left\{1-\frac{2}{m-1}\left(\Gamma\frac{m}{2}\Big/\Gamma\frac{m-1}{2}\right)^2\right\}\sigma_i^2+\ldots.$$

Therefore the variance of s_i is

$$\left\{1-\frac{2}{m-1}\left(\Gamma\frac{m}{2}\Big/\Gamma\frac{m-1}{2}\right)^2\right\}\sigma_i^2,$$

which, if 4 is substituted for m, equals $0{\cdot}151\,\sigma_i^2$, and the mean value of the standard error is $\left(\frac{2}{m-1}\right)^{\frac{1}{2}}\left(\Gamma\frac{m}{2}\Big/\Gamma\frac{m-1}{2}\right)\sigma_i$, which, if 4 is again substituted for m, equals $0{\cdot}921\,\sigma_i$. Therefore, if the regression value S_i is taken as the mean value of the standard error for a given value of X_i, $S_i=0{\cdot}921\,\sigma_i$, so that $1{\cdot}086\,S_i$ can be used as an estimate of σ_i. Thus an estimate of the variance of s_i will be

$$0{\cdot}151(1{\cdot}086\,S_i)^2=0{\cdot}178\,S_i^2.$$

Therefore,

$$\operatorname{var} b=0{\cdot}178\frac{\Sigma\,S_i^2(X_i-\bar{X})^2}{\{\Sigma\,(X_i-\bar{X})^2\}^2}=0{\cdot}178\frac{\Sigma\{\bar{s}+b(X_i-\bar{X})\}^2(X_i-\bar{X})^2}{\{\Sigma\,(X_i-\bar{X})^2\}^2}$$

$$=0{\cdot}178\left\{\frac{\bar{s}^2}{\Sigma\,(X_i-\bar{X})^2}+2\bar{s}b\frac{\Sigma\,(X_i-\bar{X})^3}{\{\Sigma\,(X_i-\bar{X})^2\}^2}+b^2\frac{\Sigma\,(X_i-\bar{X})^4}{\{\Sigma\,(X_i-\bar{X})^2\}^2}\right\}=0{\cdot}000345.$$

The standard error of b is, therefore, 0·0186, which is exceeded by the value of b nearly nine times.

For considerations given below it is necessary to know whether or not the regression equation does approximately show the relationship between standard errors and means of populations. Currently used tests for linearity of regression, based on normal distribution of variates around their regression values with the same variance over the whole range, cannot be used in this case, because the variance of s increases with the increasing X. However, a test can be made to see whether deviations of s's from their regression values are compatible with the assumption that their means lie on the regression line. If the assumption were true, mean square deviation of the values $s_i\frac{\bar{X}+c}{X_i+c}$ $\left(\text{where } c=\frac{\bar{s}}{b}-\bar{X}\right)$ from $\bar{s}$ $(=23{\cdot}1)$ should give an estimate of $0{\cdot}151\,\sigma^2$ independently of the estimate of the same value given by $0{\cdot}178\,\bar{s}^2$:

$$\frac{1}{n-2}\Sigma\left(s_i\frac{\bar{X}+c}{X_i+c}-\bar{s}\right)^2=\frac{1}{118}\Sigma\left(s_i\frac{\bar{X}+c}{X_i+c}-\bar{s}\right)^2=90{\cdot}2$$

and

$$0{\cdot}178\,\bar{s}^2=95{\cdot}0.$$

The values of the two estimates (which should be considered as based on 118 and 360 degrees of freedom, respectively) are so close to each other that obviously there is no reason to suspect that the regression equation does not give approximately the relationship between the standard errors and the means of the populations. If the regression is actually curvilinear it can be assumed that the regression curve does not deviate greatly from the straight line $S=\bar{s}+b\,(X-\overline{X})$.

Distribution of logarithmic functions of numbers of lesions

The results of considerations given in the preceding section show that numbers of lesions formed on separate half-leaves cannot be used directly for statistical analyses. Their distribution around the common mean for all plants differs considerably from normal, and variation between different plants is much greater than variation between different half-leaves within each plant. If variation within each plant is considered separately it appears that numbers of lesions obtained on separate half-leaves on different plants should be considered as samples drawn from populations with different means and different variances, although distribution of the numbers within the populations probably does not differ greatly from normal. Statistical analyses based on the assumption that all these populations have the same variance are not, therefore, applicable. In order to apply such analyses, the numbers of lesions should first be transformed so that by using some function of the numbers, instead of the numbers themselves, the variance is made the same for all the populations. It is also essential that distribution of such functions within the populations should not differ greatly from normal.

When the ratio of the standard error to the mean is constant the transformation of data into logarithms is commonly used (Cochran, 1938). By converting unequal differences between proportional values into equal differences between logarithms, this makes the standard error independent of the mean. However, with the numbers of lesions the relationship between the standard error and the mean is expressed approximately by the regression formula $S=\bar{s}+b\,(X-\overline{X})$. This means that the ratio of the standard error to the mean,

$$\frac{S}{X}=b\left(1+\frac{c}{X}\right),\quad\left(\text{where } c=\frac{\bar{s}}{b}-\overline{X}\right),$$

is not constant, but decreases with the increasing mean. Therefore the transformation of the numbers of lesions into logarithms, instead of making the standard error independent of the mean, will be expected to make it decrease with the increasing mean. On the other hand, the ratio $S/(X+c)=b$ is constant, so that the transformation $y=\log_{10}(x+c)$ (where x is the number of lesions) will be expected to be satisfactory.

Geometrically, the value of c is equal to the distance between the origin and the point of intersection of the regression line with the axis of X (see Fig. 4A). This is

equal to $\bar{s}/b - \bar{X} = 41$, so that addition of 41 to each number of lesions is equivalent to shifting the origin to the point of intersection.

Actually the value of c lies within some 'fiducial limits' which can be obtained by taking into consideration variation in $\bar{s}$ and in b of the regression equation. Both these values can be considered as normally distributed about their means. An estimate of variance of $\bar{s}$ is

$$\text{var}\,\bar{s} = \frac{0{\cdot}178}{n^2}\Sigma S_i^2 = \frac{0{\cdot}178}{n^2}\Sigma\{\bar{s} + b(X_i - \bar{X})\}^2 = 0{\cdot}178\left\{\frac{\bar{s}^2}{n} + \frac{b^2}{n^2}\Sigma(X_i - \bar{X})^2\right\} = 0{\cdot}88.$$

Therefore the standard error of $\bar{s}$ is 0·938. The standard error of b was previously found to be 0·0186. Variation of b within the limits of twice its standard error would contribute relatively much more to the variation in the value of c than a similar variation in the value of $\bar{s}$. If the latter is disregarded, fiducial limits for c based on variation in b only will not greatly differ from those that would be obtained if variation in both b and $\bar{s}$ were taken into account. Fiducial limits for b, obtained by adding and subtracting twice its standard error from the value of b actually found, are 0·124 and 0·198. These will correspond to fiducial limits for c of 14·4 and 84. Thus the value of the constant c to be used lies somewhere between 15 and 84.

To test the effect of suggested transformation of data, distributions of the following functions will be compared:

$$y = \log_{10} x, \quad y = \log_{10}(x+25) \quad \text{and} \quad y = \log_{10}(x+40).$$

The first two decimal figures were used. The constant 25 was chosen for comparison with 40 as it still lies within the 'fiducial limits', but is near to the lower limit. The results obtained with each of the three functions are summarized in Tables 3, 4 and 5. The procedure of obtaining the values given in the Tables was the same as that used above with unconverted numbers of lesions.

TABLE 3. *The values of the measures of departure from normality*

Transformation	g_1	S.D. of g_1	g_2	S.D. of g_2	G_1	S.D. of G_1	G_2	S.D. of G_2
$y = \log_{10} x$	−1·45	0·111	4·06	0·222	−0·63	0·181	0·758	0·803
$y = \log_{10}(25+x)$	−0·34	0·111	−0·18	0·222	−0·38	0·181	0·187	0·803
$y = \log_{10}(40+x)$	−0·23	0·111	−0·33	0·222	−0·37	0·181	0·186	0·803

The standard deviations are those of normal samples of the same sizes.

The values of g_1 and g_2 (Table 3) show that the distribution of the function $y = \log x$ around its mean value for all the plants is strongly skew (negatively) and strongly 'leptokurtic' (see also Fig. 1B). On the other hand, the distributions of the functions $y = \log(x+25)$ and $y = \log(x+40)$ around their means for all the plants, are only slightly skew (see also Fig. 1C and D).

The results of analysis of variance of the three functions, given in Table 4, all show very high ratios of variance between plants to that within plants, which again shows that plants differ considerably from one another in their response to inoculation with the same virus preparation.

TABLE 4. *Analyses of variances*

Transformation		D.F.	Sums of squares	Mean square (variance)	Variance ratio
$y=\log_{10} x$	Between plants	119	31·427	0·2641	18·1
	Within plants	360	5·266	0·0146	
	Total	479	36·693	0·0766	—
$y=\log_{10}(25+x)$	Between plants	119	15·252	0·1282	16·4
	Within plants	360	2·816	0·0078	
	Total	479	18·068	0·0377	—
$y=\log_{10}(40+x)$	Between plants	119	11·556	0·0971	16·2
	Within plants	360	2·165	0·0060	
	Total	479	13·721	0·0286	—

TABLE 5. *Regression coefficients of standard errors for each plant on the mean numbers of lesions per leaf in the same plant*

Transformation	Regression coefficient	Standard error
$y=\log_{10} x$	-38×10^{-5}	$7{\cdot}9\times10^{-5}$
$y=\log_{10}(25+x)$	$-4{\cdot}1\times10^{-5}$	$6{\cdot}2\times10^{-5}$
$y=\log_{10}(40+x)$	$+2{\cdot}0\times10^{-5}$	$5{\cdot}7\times10^{-5}$

From the values of G_1 and G_2, shown in Table 3, it can be seen that the distribution of deviations of the functions from their mean values in each separate plant is skew for the function $y=\log x$, but only slightly skew for the functions $y=\log(x+25)$ and $y=\log(x+40)$ (see also Fig. 3).

Table 5 shows that the coefficient of regression of standard error of $y=\log x$ for each plant, on the mean number of lesions per leaf on the same plant, is significantly smaller than zero, so that it can be concluded that samples of four values of y from each plant are drawn from populations with different standard errors, which decrease with increasing population means, as it was, indeed, expected *a priori*. On the other hand, the regression coefficients for the functions $y=\log(x+25)$ and $y=\log(x+40)$ are smaller than their standard errors, and, therefore, cannot be considered as significantly different from zero. Comparison of 'dot diagrams' on Fig. 4 B, C and D show the differences in distribution of the standard errors for the three functions.

The conclusion is that the function $y=\log x$ is not suitable for use for exactly the same reasons as untransformed numbers of lesions. On the other hand a function $y=\log(x+c)$ can be used satisfactorily, for its distribution around the common mean for all plants does not differ greatly from normal, and its values obtained on separate plants can be considered as samples from populations not greatly differing from normal with the same variance, although with different means.

In practice the line of regression of standard errors of numbers of lesions obtained on different plants on the mean numbers of lesions on the same plants can be fitted approximately and the value of a constant c to be used thus obtained. There is no

need for great accuracy, for the value of the constant lies within a rather wide range of probable values and using any number within the range seems to give satisfactory results. If there are not enough data for doing this, a value of between 5 and 20 can be used as a constant. It is not likely that this will lead to over-correcting.

So far only variations between half-leaves on all plants, between plants and within plants have been considered. It is obvious that the plants differ greatly in susceptibility. In order to design experiments properly it is necessary to know whether there are any other sources of variation. The results of the analysis of variance within plants, based on the transformation $y = \log(x+40)$ and given in Table 6, show that

TABLE 6. *Analysis of variance within plants (based on the transformation* $y = \log_{10}(x+40)$)

		D.F.	Sums of squares	Mean square (variance)	z	P (approx.)
Between leaves		120	0·984	0·0082	0·2667	0·1 %
Within leaves	Left-right	1	0·032	0·032	0·9522	1 %
	Residual	239	1·149	0·0048	—	—
	Total	240	1·181	0·0049	—	—
Within plants (total)		360	2·165	—	—	—

variation between different leaves on each plant and also the difference between all left and all right half-leaves, are both significantly greater than could be expected from chance variation between half-leaves on each leaf. Thus leaves belonging to the same plant can differ in susceptibility and there is a tendency for more lesions to be formed on the left than on the right half-leaves. This probably results from the inoculation technique and, therefore, can vary from one experimenter to another.

Therefore, if four different virus preparations are to be compared, each should be inoculated into one of four half-leaves on a number of plants, each should be paired on the same leaf with every other an equal number of times and each should occur on an equal number of left and right half-leaves. An essentially similar experimental design can be made when two or three preparations are to be compared. When more than four preparations are to be compared, an experimental design of the 'balanced incomplete blocks' (see Fisher & Yates, 1948) can be applied or blocks containing as few plants as possible can be arranged. If possible, variation between plants should be completely eliminated by means of analysis of variance, but with some experimental designs, when more than four preparations are compared, it may be possible to eliminate it only partially. Then it may become of some importance that the distribution of transformed numbers of lesions should not differ greatly from normal not only on separate plants but also on all plants as a whole. The transformation $y = \log(x+c)$ fulfils this condition.

Results of only one experiment are here discussed in detail, but other experiments with a tobacco necrosis virus on beans and also with tobacco mosaic and tomato bushy stunt viruses on *Nicotiana glutinosa* gave essentially similar results. There is,

therefore, reason to believe that the conclusions reached here apply generally. With *N. glutinosa* the problem is more complicated because it has up to eight or ten inoculable leaves which differ from each other in age, in their position on the plant and in susceptibility. As proposed by Youden & Beale (1934), variation between different plants and between different leaf positions can be eliminated by applying the experimental design of Latin square and by analysis of variance. It should, however, be borne in mind that the choice of leaves to be inoculated is to a large degree arbitrary, so that correspondence between leaves in the same position on different plants is somewhat artificial, and that relationship between leaf position and susceptibility can vary from plant to plant.

It must be pointed out that mean numbers of lesions per half-leaf, obtained with any treatment, should not be smaller than 5–10. When means of some treatments are smaller than this, i.e. when a proportion of half-leaves have no lesions, the transformation $y = \log_{10}(x+c)$ may not make the standard errors independent of the means.

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(*Received* 1 *May* 1948)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 1 October 1948, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, n the Chair.

Nutrition in relation to plant disease

The following papers were read and discussed:

1. Some effects of host nutrition on susceptibility to plant viruses. By Mr F. C. Bawden.
2. Some fruit diseases. By Mr M. H. Moore.
3. Experiments on the relationship of plant nutrition to *Verticillium* wilt of the hop. By Dr W. G. Keyworth.
4. *Verticillium* wilt of the hop: some problems and difficulties. By Dr N. H. Pizer.

Ordinary Meeting of the Association held on Friday, 5 November 1948, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

The insecticidal properties of certain organo-phosphorus compounds

The following papers were read and discussed:

1. Introduction. By Dr H. Martin.
2. The chemistry of phosphorus insecticides. By Mr H. Coates.
3. Preliminary experiments with systemic insecticides. By Mr S. H. Bennett.

THE INSECTICIDAL PROPERTIES OF CERTAIN ORGANO-PHOSPHORUS COMPOUNDS

By Hubert Martin, *Research Station, Long Ashton, Bristol*

The organic chemistry of phosphorus has been of slow growth except for the biochemistry of phosphorylation which is largely concerned with the reactions of inorganic phosphate in metabolic processes. The pioneer work of August Michaelis (1903), published before 1903, was mainly on the aromatic and aliphatic phosphines though in his next contribution, twelve years later (Michaelis, 1915), he dealt with some phosphine oxides. But Michaelis's work, to which Schrader (1947) pays tribute, doubtless encouraged the latter to pursue a systematic survey of the organo-phosphorus compounds in the course of which instances of astonishing physiological activity were discovered.

The simple esters of pyrophosphoric acid had previously been prepared by Balareff (1914) and by Nylén (1930) in studies on the structure of this acid, but no mention of physiological properties of these esters was made by these workers. Schrader repeated these syntheses and with his colleague Kükenthal found that aqueous solutions of tetraethyl pyrophosphate were toxic to aphids at a dilution of 0·001 %, an extreme dilution when it is

remembered that nicotine is used for aphid control at fifty times this concentration. Tetraethyl pyrophosphate is, however, extremely unstable in aqueous solution which Schrader found lost half its insecticidal potency in 2 hr.; for this reason and because of its high toxicity to man, he did not consider the ester suitable for development as a commercial insecticide. By the interaction of phosphorus oxychloride (1 mol.) with triethylphosphate (3 mols.) he obtained a product which, from its method of preparation and its analysis, he regarded as hexaethyl tetraphosphate (D.R.P. 720, 570). This compound, often called HETP, though of lower insecticidal potency than tetraethyl pyrophosphate, he concluded was stable enough to water to justify development. It was the effective component of the proprietary product Bladan first officially approved in Germany in 1944 for use against aphids.

The value of hexaethyl tetraphosphate as a substitute for nicotine in aphid control has been established by extensive trial both here and in the States; one remaining point of contention is whether or not its susceptibility to hydrolysis is a serious hazard in its practical use. Careless handling such as transference to wet containers or delays in applying the diluted material would certainly lead to unsatisfactory results; is there evidence that such misuse is common enough to make it unwise to recommend the product to growers? Further, if growers can be taught the proper use of hexaethyl tetraphosphate, might not the more potent but more dangerous tetraethyl pyrophosphate be used instead?

From the chemical aspect, hexaethyl pyrophosphate is fascinating material; it seems most unlikely that the tetramolecular reaction leading to the nicely symmetrical structure ascribed to it by Schrader can be realized under the conditions of the synthesis. To test the point, Bennett and I (1947) examined samples of the reputed compound prepared by different methods. We used bioassay methods which should have revealed differences in the rates of hydrolysis of the samples, but could detect none. Yet there is now abundant physicochemical evidence that the material is a mixture, the most plausible hypothesis being that it is an equilibrium mixture of ethyl polyphosphates of which tetraethyl pyrophosphate is the most active member.

Ready hydrolysis to non-toxic products, although an advantage from the spray residue aspect, precludes the use of the ethyl polyphosphates in alkaline media such as lime sulphur. Consequently, Schrader devoted much time to the discovery of lime-stable phosphorus insecticides. He found that in general the thiophosphates were less susceptible to reaction with water than the corresponding phosphates. The tetraethyl ester of thio-pyrophosphoric acid, for example, he found stable to mild alkali and to give a complete kill of aphids at 0·005 %. But no further investigations of this compound have yet been published, though from Schrader's figures it would not appear to be highly toxic to warm-blooded animals. Schrader (1948) considered that the toxophores of these compounds are the $(RO)_2PX.O^-$ and $(R_2N)_2PX.O^-$ groups where $X=O$ or S, and, by a systematic study of the diethyl thiophosphates, he was led to E 605, *p*-nitrophenyl diethyl thiophosphate, a compound of the same order of toxicity to aphids as tetraethyl pyrophosphate (0·001 % giving 100 % kill) but of high potency to a much wider range of insects. The compound has not been widely studied in this country, but the limited tests so far published confirm the German work and, in addition, it has been found at Long Ashton to possess interesting ovicidal properties, inhibiting the hatch of aphis and winter moth eggs at concentrations of 0·05 % or less. The claim for true ovicidal properties, although made originally by the German workers (Martin & Shaw, 1946), has since been questioned by them (Schrader, 1948). Before insecticides based on E 605 can be recommended to growers for use on food crops it would seem advisable to check reports (Unterstenhöfer, 1948) that the compound is absorbed by plant tissue. If true, the problem of residue, for the compound is highly toxic to man, becomes complicated.

The ability to penetrate into plant tissue and to be translocated within the plant is, however, clearly shown by certain other of Schrader's compounds. One such compound is bis(dimethylamino)fluorophosphine oxide $[(CH_3)_2N]_2POF$, a derivative of the N–P type first examined by Michaelis. This compound, first prepared by Schrader in 1940–1, is stable to and soluble in water, and when solutions at 0·02–0·05 % are watered around the roots of

plants, the plants were found to become systemically insecticidal. Schrader called this property a chemo-therapeutic effect; but as this term has been applied to all spray chemicals by Cunningham (1935), Shaw and I (1946) preferred to borrow from pathology the term 'systemic'. This property is also found in the pyrophosphate derivative

$$[(CH_3)_2N]_2PO.O.PO[N(CH_3)_2]_2,$$

the tetrakis dimethylamide of pyrophosphoric acid, according to Schrader to a greater degree than in the fluorophosphorine oxide, though Bennett has not substantiated this claim. In view of the many previous failures of this type of chemo-therapeutic pest control in plants, it was interesting to learn that soil application was included by Kükenthal in the routine biological examination of Schrader's compounds. The systemic property seems to have been discovered first, about 1940, in a phosphorus-free compound, bis(betafluoroethoxy)methane $CH_2(OCH_2CH_2F)_2$. But this compound, like the two phosphorus compounds already mentioned, renders the treated plant toxic, for a period at least, to human beings. Consequently, little work on the commercial application of their discovery seems to have been undertaken by Schrader and his associates. Yet the discovery is of such obvious academic interest, providing a new approach to the problems of translocation in plants, that it has been followed up at Long Ashton.

It should, however, be emphasized that this Long Ashton work was not undertaken with the object of any practical application of this systemic property. Clearly a knowledge of the mechanism by which the poison is taken up by and moves within the plant would facilitate work on non-poisonous systemic insecticides, but the known systemic insecticides are so closely related to compounds of high and dangerous physiological activity that the greatest caution must be taken before any are recommended for practical use. Hydrolysis of the fluoroethoxymethane, for instance, would give fluoroethyl alcohol, whose physiological activity has been described by McCombie & Saunders (1946*b*). Test animals exposed to concentrations of 0·1 g./m.^{3} for 10 min. were apparently unaffected, but within an hour had died with violent convulsions. Although the fluorophosphine oxide is probably stable, the extreme toxicity of the related alkyl fluorophosphonates was found by McCombie & Saunders (1946*a*); a 5 min. exposure of human beings to a concentration of 1 p.p.m. caused severe miosis and loss of powers of visual accommodation for several days; amounts too low to be detectable chemically caused a blurring of vision. The pyrophosphoryl tetrakis dimethylamide would seem less likely to give rise to such unpleasant compounds, but clearly the fate of the compound within the plant tissue must be known before its use could be recommended to growers. But it should be recorded that contact with these compounds and with plants containing them has not apparently affected us at Long Ashton.

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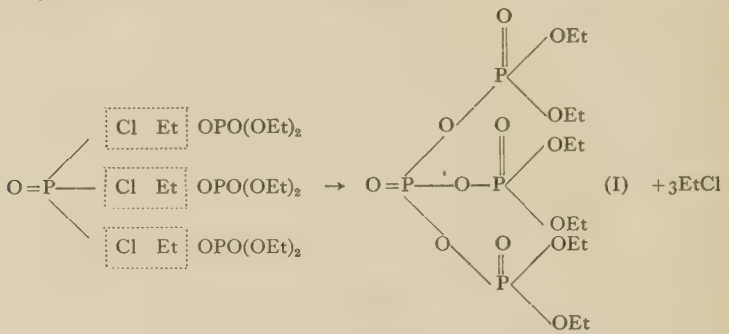
THE CHEMISTRY OF PHOSPHORUS INSECTICIDES

By H. Coates, *Messrs Albright and Wilson, Ltd., Oldbury, Birmingham*

The first organo-phosphorus compound to attain any prominence as an insecticide was the material sold in Germany during the war as a nicotine substitute under the name 'Bladan'. This material was reputed to be hexaethyltetraphosphate and was given the structure (I) by the discoverer, Dr G. Schrader, of I.G. Farbenindustrie.

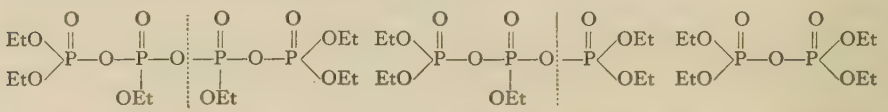


This was originally prepared by Schrader by heating a mixture of phosphorus oxychloride (1 mole) and triethyl phosphate (3 moles) to a temperature of approximately 140°. Three moles of ethyl chloride were eliminated. (B.I.O.S. 714, 1095 and 1808.)

When triethyl phosphate is not available the same product may be prepared by reaction of ethyl alcohol, and phosphorus oxychloride according to the equation (FIAT 949):

$$9\,EtOH + 4POCl_3 \rightarrow (EtO)_6P_4O_7 + 9HCl + 3EtCl.$$

Bladan or H.E.T.P. appears in fact to consist of a mixture of the linear polyphosphoric esters up to and including hexaethyltetraphosphate, i.e.



together with some unreacted triethyl phosphate and acidic by-products. That no appreciable quantity of material possessing the branched chain structure (I) is present is shown by the fact that only a very small amount of orthophosphoric acid is produced on complete hydrolysis. The formation of these linear compounds is explained by the fact that on heating a mixture of triethyl phosphate and phosphorus oxychloride a disproportionation takes place with the formation of the chloro compounds $(EtO)_2POCl$ and $EtO.POCl_2$ (McCombie, Saunders & Stacey, 1945). On heating to 140° these chloro bodies react with unchanged triethyl phosphate to give the mixture of polyphosphoric esters outlined above. The fact that this mixture analyses closely for hexaethyltetraphosphate is to be expected, since the required amount of ethyl chloride is eliminated in the reaction.

An alternative process for the manufacture of H.E.T.P., patented in America by Woodstock (U.S. P. 2, 402, 703), consists of heating a mixture of phosphorus pentoxide and triethyl phosphate to 140–160°, e.g.

$$P_2O_5 + 2(EtO)_3PO \rightarrow (EtO)_6P_4O_7.$$

It is significant that materials prepared by all three processes using the same reaction times

and temperatures appear to be identical to all intents and purposes, and this leads one to believe that the material is in effect an equilibrium mixture.

Schrader and others concluded that the active principle of H.E.T.P. is tetraethyl pyrophosphate, a compound which in the pure state is some five times as potent as H.E.T.P. It is becoming current practice to refer to commercial material containing approximately 20% of tetraethyl pyrophosphate as H.E.T.P. At or above 40% pyro ester content the material is designated T.E.P.P.

Tetraethyl pyrophosphate

There are two well-known methods for preparing this compound.

(1) By reaction of silver pyrophosphate and ethyl iodide under reflux. It is essential in this preparation that the reagents be perfectly dry. This method is obviously not commercially practicable owing to the large amount of silver needed, although it would probably be the best method for preparing tetraethyl pyrophosphate containing P^{32}. As tetraethyl pyrophosphate is somewhat sensitive to heat and traces of impurities during distillation, it is essential to distil under a high vacuum, e.g. $<0{\cdot}1$ mm., to obtain a pure product.

(2) An alternative method, which is much more convenient, consists in reacting ethyl alcohol with phosphorus trichloride at 0–10° to give diethyl hydrogen phosphite, followed by chlorination of this material, at the same temperature giving diethyl chlorophosphonate in good yield (McCombie *et al.* 1945):

$$3EtOH + PCl_3 \longrightarrow 2HCl + EtCl + (EtO)_2POH \xrightarrow{Cl_2} (EtO)_2POCl + HCl.$$

This material on reaction with excess triethyl phosphate at 140° gives T.E.P.P.

As tetraethyl pyrophosphate is considered to be the main active ingredient in commercial H.E.T.P. and T.E.P.P., it is essential to have a reasonably accurate method for determining the amount of this material present in such mixtures. The methods available (Hall & Jacobson, 1948; Dvornikoff & Morrill, 1948) depend on the relative rates of hydrolysis of the various esters. It is found that hexaethyltetraphosphate and pentaethyl triphosphate are split on hydrolysis very rapidly at the positions marked by the dotted lines (see p. 156). The derivatives of pyrophosphoric acid thus formed, and also tetraethyl pyrophosphate itself, then undergo hydrolysis at a much slower rate. By carrying out a time titration on separate samples, using methyl orange and phenolphthalein as indicators, one may calculate the amount of tetraethyl pyrophosphate present in the mixture. This is more convenient than the procedures outlined in the American references cited above, which involve extraction of the material with chloroform or benzene. The results by the two methods agree to within 2% or less. It is important to note that in the hydrolysis of pure tetraethyl pyrophosphate in the cold, no appreciable amount of alcohol is formed.

From the agricultural and insecticidal point of view one of the most important properties of a compound is its rate of hydrolysis under field conditions. Some kinetic measurements of the hydrolysis of tetraethyl pyrophosphate and H.E.T.P. by D. R. Peck, N. H. Poynton and B. Topley were reported at a meeting of the Physiological Society in December 1947 (Peck, 1948).

Tetraethyl pyrophosphate is soluble in water and at the concentrations normally employed the hydrolysis is found to be first order provided the pH is either constant or else on the acid side. We thus have

$$k = \frac{1}{t}\log_e \frac{a}{a-x} = \frac{2{\cdot}303}{t}\log_{10}\frac{a}{a-x},$$

where a = amount initially present; x = amount used after time t; k = the velocity constant, in reciprocal minutes; t = time in minutes.

It was anticipated that k (for hydrolysis at fixed pH) would be made up of at least three effects, as follows:

$$k = K_{H^+}[H_3O^+] + K_{OH^-}[OH^+] + K_{H_2O}[H_2O]\ldots, \text{ etc.}$$

It was found that the hydrolysis was not appreciably catalysed by hydrogen ions and that k was, for practical purposes, the sum of two terms, i.e.

$$k = K_{OH}[OH^-] + K_{H_2O}[H_2O].$$

As the concentration of the water is constant at 55·5 mol./l.,

$$k = K_{OH}[OH^-] + k_{H_2O},$$

where $[OH^-]$ is the molarity of OH^-. At 25° for tetraethyl pyrophosphate

$$k = 160[OH^-] + 1{\cdot}6 \times 10^{-3} \text{ min.}^{-1}.$$

It is convenient to express the rate of hydrolysis in terms of the time for 50% decomposition:

$$t_{50\%} = \frac{\log_e 2}{k} = \frac{0{\cdot}693}{k}.$$

It will be seen that even under conditions of maximum hydrolytic stability (pH 6 or less) the time for 50% decomposition is only 7 hr. at 25° C. At 15° C. this time becomes 15 hr.

In view of the pronounced instability to water of T.E.P.P. it was natural that one should investigate the sulphur-containing analogues. One such is tetraethyl monothionopyrophosphate first prepared by Schrader:

$$(EtO)_2P(:S)OP(:O)(OEt)_2. \quad \text{(II)}$$

This compound is prepared by reacting the sodium derivative of diethyl hydrogen phosphite with sulphur as described by Foss (1947) to give sodium diethylthiophosphate which is then reacted in an inert solvent with diethyl chlorophosphonate, giving the thionopyro compound in good yield. The solubility of this compound in water (0·06% at 25°) is much lower than that of tetraethyl pyrophosphate and this necessitated measuring the hydrolysis in 20% alcohol solution. A comparison at 25° C. and pH 10 with tetraethyl pyrophosphate in the same medium gave

	In 20% alcohol	In water
Tetraethyl pyrophosphate	$16{\cdot}3 \times 10^{-3}$	$17{\cdot}5 \times 10^{-3}$
Tetraethyl monothionopyrophosphate	$3{\cdot}68 \times 10^{-3}$	—

We see that the rate of hydrolysis of the monothiono pyro ester is approximately one-fifth that of tetraethyl pyrophosphate under similar conditions, giving as the time for half decomposition about 1½ days at 25° C., when the solution is allowed to become acid during the hydrolysis.

The dithionopyrophosphate may be prepared in an analogous manner. Hydrolysis data for this compound are not yet available. The anticholinesterase effect of the monothiono body appears to be of the same order as that of tetraethyl pyrophosphate.

In the search for other compounds of the phosphoric ester series more stable than tetraethyl pyrophosphate Schrader prepared diethyl *p*-nitrophenyl phosphate (E 600). This is very readily prepared by reaction of diethylchlorophosphonate with anhydrous sodium nitrophenate in an inert medium. The product is a pale yellow oil, soluble in water to the extent of approximately 0·2%.

The hydrolysis of this compound produces the nitrophenate ion and may be followed colorimetrically. In this case it was found that the uncatalysed reaction with water is very small and the overall velocity constant is

$$k = 0{\cdot}52[OH^-] + 1 \times 10^{-6} \text{ min.}^{-1},$$

showing that E 600 is *roughly* 300 times more stable to hydrolysis than tetraethyl pyrophosphate. It is also believed to be more toxic to insects. Its use as an insecticide appears to have been abandoned owing to its toxicity to warm-blooded animals, although Schrader stated that he had made a small quantity for use in the treatment of *Myasthenia gravis*.

The next compound to receive serious consideration was the thiophosphate corresponding to E 600, i.e. O:O-diethyl O:*p*-nitrophenylthiophosphate (E 605 or Parathion). This is prepared by reacting thiophosphoryl chloride $PSCl_3$ with sodium ethoxide (2 moles) to give diethoxythiophosphoryl chloride $(EtO)_2PSCl$ which is then reacted with anhydrous sodium nitrophenate in xylene or chlorobenzene at 120–130°. The product is a pale yellow oil, very sparingly soluble in water. The solubility in water at 25° C. is 15–20 p.p.m.

An attempt was made to study the hydrolysis of this compound in the form of an emulsion, but the results could not be interpreted. The hydrolysis was followed colorimetrically using an aqueous solution of the pure material in caustic soda solution ranging from 0·0001 to 0·5 *N*. Again there was no catalysis by hydrogen ions and the uncatalysed water had only a very small effect. The overall velocity constant is

$$k = 0{\cdot}047\ [OH^-] + 4 \times 10^{-6} \text{ at } 25°,$$

giving a time for half decomposition of some 120 days, in the absence of alkali (Peck, 1948).

Thus we see that the insecticides discussed form a series with progressively increasing stability to hydrolysis, i.e.

$$\text{E 605} > \text{E 600} > \text{Monothionopyrophosphate} > \text{T.E.P.P.} \cong \text{H.E.T.P.}$$

Much interest has been aroused, since the publication of the various *B.I.O.S. Reports*, in a series of organo-phosphorus compounds which have been shown by Schrader and other workers to be systemic insecticides. Prominent amongst these compounds are bisdimethylaminofluorophosphine oxide (III) and pyrophosphoric tetrakis-(dimethylamide) (IV).

$(Me_2N)_2POF$ (III) $\qquad$ $(Me_2N)_2P(:O).O.P(:O)(NMe_2)_2$. (IV)

(III) may be prepared by two methods. The first consists in reacting phosphoryl dichlorofluoride $POCl_2F$ with 4 moles anhydrous dimethylamine in an inert solvent

$$POCl_2F + 4Me_2NH \rightarrow (Me_2N)_2POF + 2Me_2NH.HCl.$$

This method is not practicable on a large scale owing to the poor overall yield on $POCl_3$.

A more convenient method consists in reacting phosphorus oxychloride with 4 moles anhydrous dimethylamine in benzene or petrol to give bisdimethylaminochlorophosphine oxide $(Me_2N)_2POCl$, which is then reacted with sodium fluoride to give (III). An advantage of this procedure is that the dimethylamine hydrochloride formed as a by-product may be reworked by refluxing with an excess of $POCl_3$ to give

$$Me_2N.POCl_2, \text{ e.g. } Me_2NH.HCl + POCl_3 \rightarrow Me_2N.POCl_2 + 2HCl$$

(Michaelis, 1903). This by further reaction with dimethylamine, followed by sodium fluoride, gives more (III). Hydrolysis figures on this compound are given by Saunders (Heap & Saunders, 1948).

The compound IV may be prepared from $(Me_2N)_2POCl$ by hydrolysis and conversion to the sodium salt followed by reaction of this anhydrous sodium salt with one mole of the chloro compound in toluene, e.g.

$$(Me_2N)_2P(:O)ONa + ClOP(NMe_2)_2 \rightarrow (Me_2N)_2P(:O).O.P(:O)(NMe_2)_2.$$

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PRELIMINARY EXPERIMENTS WITH SYSTEMIC INSECTICIDES

By S. H. Bennett, *Research Station, Long Ashton, Bristol*

The term 'systemic insecticides' refers only to chemical substances which are absorbed by the plant and translocated to all parts of it, rendering it insecticidal. Prior to these experiments one might have defined them as 'materials rendering the plant toxic to insects *feeding* upon it', but as will be shown, this definition is not strictly correct.

Many attempts have been made in the past to find such materials, and until recently the most promising were compounds of selenium. This work originated in the observations of Hurd-Karrer & Poos (1936) that wheat growing on seleniferous soils was not attacked by aphids. Since that time many applications of this property of selenium compounds have been made.

Because of its close chemical relationship to sulphur—an element essential to plant growth—the uptake of selenium by plants is not surprising. From the work of Hurd-Karrer (1937) it may be assumed that selenium compounds can replace sulphur in the plant metabolism and are absorbed and translocated in selenium-tolerant plants in the same way as the sulphur compounds. It is more difficult to visualize a mechanism for the absorption and translocation of a bulky insecticidal molecule foreign to the plant. Yet there was evidence that the movement of such a molecule within the plant is possible, for Fulton & Mason (1937) found that if derris is applied to the first true leaves of bean plants, the leaves formed subsequently are less readily attacked by larvae of the Mexican bean beetle. This work has never been repeated or extended, however, and so the claims of Schrader (1947) that certain of his insecticides were capable of absorption by plants which thereby became systemically insecticidal seemed almost incredible.

Schrader found this property among widely separated types of compounds; the most effective being, on the one hand, certain derivatives of fluoroethyl alcohol and, on the other, certain alkyl amino phosphorus compounds.

Unfortunately, the substances claimed by Schrader to be most successful were not available, but in 1947 a sample of bis(2-(fluoro-ethoxy) methane $CH_2(OCH_2CH_2F)_2$ was obtained for preliminary experiments and this year two other materials, the bis(dimethylamino) fluorophosphine oxide $[(CH_3)_2N]_2POF$ and a commercial preparation based on tetra (dimethyl amido) pyrophosphate $[(CH_3)_2N]_2PO.O.PO[N(CH_3)_2]_2$.

The experiments that have been carried out have been designed to try and find out a little about the action of these materials and have no applied significance at all. Most of the materials—in fact all of them under certain conditions—are highly phytotoxic and no effort has been made to use them at concentrations below the phytotoxic level. It is felt to be highly desirable to find out far more about their mode of action, and the residual effect both in the plant and the soil before they are applied to general crops.

Before mentioning experiments carried out with the organic compounds there are one or two points about selenium which are worth mentioning here. In one experiment wheat was watered during the flowering stage with aqueous solutions of fluoroethoxy methane and sodium selenate. The grain was harvested at maturity and then infested with adult grain weevils of *Calandra granaria*. Four weeks after, the adults were removed and counted, the grain stored for another 4 weeks when the number of adult weevils present was determined (see Table 1). This table shows that neither the selenate nor the fluoro-ethoxy methane treatments have given grain directly toxic to the adult weevils. That no infestation is built up on the selenate-treated wheat suggests that the absence of eggs or larvae is the result of the selenate treatment. The presence of selenium would appear to affect the reproductive processes, possibly by derangement of sulphur-containing proteins or vitamins.

Table 1. Calandra granaria. *Infestation of grain from treated wheat*

Insecticide	Vol. (ml.)	No. of applications	No. adults added	Deaths after 4 weeks	Population after 8 weeks
0·1 % sodium selenate	10	1	56	1	429
	50	1	50	2	124
	50	2	50	1	75
	50	3	61	5	0
1·0 % fluoro-ethoxy methane	10	1	50	1	437
	10	2	51	7	174
	100	1	49	2	398
	100	2	50	2	265
Nil	—	—	44	4	344

Comparable results have been obtained by soaking wheat in aqueous solutions of sodium selenate and rearing *C. granaria* by a method similar to that described.

For the control of chrysanthemum eelworm, sodium selenate at 25–50 p.p.m. of soil has been recommended. It has been found, however, that free eelworms can live for several days in a 1·0% solution so that here again it is conceivable that the reproductive capacity of the eelworms is affected. Experiments are in hand to determine this point.

*Organic compounds**

Aqueous solutions of fluoro-ethoxy methane were applied to the soil of pots in which plants were growing and the following results were obtained.

10 ml. of a 1·0% solution were applied to a 4 in. pot in which a groundsel plant was growing. Twenty-one hours after the application larvae of the cinnabar moth were placed on the plant and in 18 hr. all were in a moribund condition: little feeding had taken place.

Infestations of *Aphis fabae* on bean plants were eliminated by the application of 1 g./8 in. pot and considerably reduced with concentrations as low as 0·05 g./8 in. pot.

With cauliflower plants growing in 10 in. pots and left in the open, it was very noticeable that the damage caused by the various leaf-eating caterpillars was considerably less than on the untreated plants—even when the treated plants received only 0·1 g. of the insecticide.

In an unheated greenhouse, cauliflower plants treated with 0·5 g. remained free from damage by *Pieris* spp. for about 3 weeks. Eggs were laid freely on the plants and the eggs hatched, but the larvae died, presumably after eating only a trace of leaf tissue.

As it seemed possible that the material might be acting as a fumigant, a laboratory test was designed primarily to establish the fact. The petioles of cabbage leaves were inserted in 3 × 1 in. specimen tubes containing water or a 1·0% aqueous solution of the fluoro-ethoxy methane. The specimen tubes were then placed in beakers containing the insecticide or water respectively so that leaves standing in water and those standing in insecticide were kept over both insecticide and water. Young larvae of *P. brassicae* were then transferred to the leaves and only those leaves with their petioles in water were eaten by the caterpillars. An effort to determine quantitatively the amount required for a lethal dose failed, as larvae of the cabbage moth refused to eat disks taken from treated leaves. Experiments this year have proved conclusively that the materials under test, F, D and P, all show a fumigant action against mealy cabbage aphis, although the action of the last-named is only slight. It was found that if the insecticide was applied to a filter-paper roughly equivalent in area to the leaf span of the plant and placed around the base of the plant, the population of aphids was greatly reduced, but a 100% control was not obtained. Plants, on the other hand, receiving the same amount of insecticide applied to the soil, which was then waxed to prevent fumigant

* The following code letters are employed: F for bis-fluoroethoxymethane, D for bis-dimethylaminofluorophosphine oxide, P for tetradimethylamidopyrophosphate.

action, were completely cleared of aphids in 3–7 days, though the initial action was much slower. Approx. 200 p.p.m. in soil kept plants free of aphis for 4·5 weeks and 40 p.p.m. clears plants of aphis. It would appear from this that when materials are applied to the soil in which plants are growing, the initial action is a fumigant one and later the material acts as a systemic insecticide.

In order to overcome the effects of fumigant action in experiments it was decided to grow plants in water solutions so that the residual fumigant effect from treated soil could be avoided. Willow sets about 6–8 in. long and approximately ½ in. in diameter were rooted in nutrient solution. Each threw out one main shoot 18–24 in. long and a few small secondary shoots. The brassy willow beetle, *Phyllodecta vitellinae*, was used as a test insect.

5 ml. of aqueous solution of the insecticide under test were placed in the lower half of a 9 cm. Petri dish over which a muslin circle was stretched. The beetles were placed on this muslin and confined by the upper half of the dish. Solutions containing as little as 50 μg. of D or F showed fumigant action against *P. vitellinae* under these conditions. D was more effective and rapid in action than F, and P had no action as a fumigant whatsoever.

In another experiment, where the set was allowed to take up the insecticide through the roots, leaves were later removed and placed with the beetles. It was noticed that the beetles died very quickly and after only a very small trace of leaf tissue had been eaten. This pointed to the possibility that the leaf tissue itself was acting as a fumigant and this later proved to be the case.

An experiment was therefore designed to check the effectiveness of this tissue as a fumigant and also to attempt to determine its effectiveness quantitatively. It was necessary to assume that if a given volume of insecticide was absorbed by the plant then the distribution of that insecticide through the plant would be equal. The time taken for the insecticide to reach the top of the plant had been determined previously, and on a bright summer day when conditions for transpiration were good it took about 4 hr. to reach the tip of an 18 in. shoot.

The set was allowed to take up a known weight of insecticide after which various parts of the plant—leaf, shoot and stem were removed. Equal weights were taken and beetles exposed to this tissue in various ways. The weights taken allowed for each treatment to contain ½ mg. of insecticide. A number of interesting points were observed (see Tables 2 and 3).

Table 2. *Toxicity of willow tissue to* Phyllodecta vitellinae

	First symptom after /hr.	100% moribund after /hr.
Leaf	1	5
Shoot	1	24
Stem	2	40
Solution	16	65

Table 3. *Toxicity of willow leaf tissue to* Phyllodecta vitellinae

	First symptom after /hr.	100% moribund after /hr.
Overall action normal humidity	1	5
Overall action high humidity	2	6
Fumigant. Leaf shredded normal humidity	3	6
Fumigant. Leaf shredded placed in water	4	48
Fumigant. Leaf whole normal humidity	1	12
Fumigant. Leaf whole placed in water	1	64
Solution 0·0005 g.	16	65
Solution 0·05 g.	1	4

Table 2 shows the overall toxicity, i.e. fumigant, contact and stomach-poison effect—where the tissue is placed with the beetles. It shows that the leaf is more toxic than the

shoot which in turn is more toxic than the stem, the last-named being more toxic than an aqueous solution of equivalent weight of insecticide.

Table 3 shows the effect of exposing the leaf tissue to the beetles in various ways. When the leaf tissue is acting as a fumigant the surface area of the material will be important.

The insecticide in leaf tissue used in this way is as effective as 100 times the weight of insecticide used in solution.

In an experiment to determine the speed of translocation of the material through the plant, leaves were placed in 3 × 1 in. tubes, beetles introduced and the tubes sealed. The root of the set was then placed in insecticide. No effect was observed on the beetles after 8 hr. yet leaves on either side of the tubed leaves, when removed from the plant, killed beetles very quickly. In this case it would seem that the atmosphere inside the tube becomes saturated with water vapour before the insecticide actually reaches the leaves and the saturated atmosphere either prevents the insecticide from reaching the leaf or the vapour being given off.

Using Lepidopterous larvae as test insects it was found that solutions containing 50 mg. of D or P were ineffective as fumigants against last-instar larvae of *Pieris brassicae*, but F was effective and the larvae were in a moribund state after 4 hr. When the overall toxicity was checked by placing 10 mg. in aqueous solutions on to filter-paper, larvae of *P. brassicae* were in a moribund condition after 4 hr. with solution F, after 48 hr. with solution D, and P was again ineffective.

Using tomato moth larvae only solution F was effective as a fumigant at 50 mg. When using tissue from plants that had absorbed solutions, however, tissue from plants treated with solution D was effective as a fumigant and showed overall toxicity.

Tissue from plants treated with solution F was not effective as a fumigant but larvae were generally repelled from leaf tissues and there were some deaths after eating only small amounts.

Tissue from plants treated with solution P had no effect on larvae at all.

Duration of toxicity

F and P applied to the soil at 200 p.p.m. will keep cauliflower plants free from infestation by mealy cabbage aphis for 3 to 4 weeks, but only D is toxic to larvae of *P. brassicae* 7 weeks after application to the soil.

All materials under some conditions are phytotoxic even at very low concentrations. D for instance, produces a marginal scorch on cauliflowers at between 20 and 50 p.p.m. of soil. P has not been found phytotoxic when applied to the soil in which plants are growing, but is so when the roots are placed directly in the solution.

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REVIEWS

List of Recorded Cotton Insects of the World. By H. HARGREAVES, A.R.C.S, D.I.C. Pp. 1–50. London: Commonwealth Institute of Entomology, 41 Queen's Gate, S.W. 7. 1948. 5*s.* 6*d.*

The author in the introduction to this list states that: 'The aim has been to bring together for quick and easy reference the names of all insects and mites recorded from cotton in literature abstracted in the *Review of Applied Entomology*, Volumes 1 to 34 inclusive.'

The list indicates the part of the plant attacked and the country or countries from which each species has been recorded. There is also a list of representative literature and a geographical index—a very useful publication for all economic entomologists concerned with cotton insects.

I. THOMAS

The Potato. A Survey of its History and of Factors Influencing its Yield, Nutritive Value and Storage. By W. G. BURTON. Pp. xiv + 319. London: Chapman and Hall, Ltd. 1948. 25*s.*

During the past thirty years the potato has been the object of much investigation. Researches have been carried out on its origin and wild relatives, on the breeding of new varieties, breeding for disease resistance, virus diseases and their epidemiology, nutritive value and cooking quality of the tubers, methods of storage, methods of processing, etc. There have also been studies of such agricultural questions as manuring of the crop, spacing of sets, cultivations, spraying, haulm-killing, and other factors affecting yield. When knowledge advances rapidly and on a wide front, in this way, there inevitably comes a time when it is worth someone's while to review the literature, sift the grain from the chaff, and set out the information properly documented for the use of his fellow workers. This is the task Mr Burton has set himself, for he describes his book as 'an attempt to summarize our knowledge of the potato as a source of food, more particularly from the viewpoint of a plant physiologist'.

Again quoting from the preface, the approach is said to be broadly as follows: 'First, what is the origin and nature of the potato? Secondly, what is its yield per acre and how is this influenced by such factors as climate, manuring, variety and disease? Thirdly, what proportion of this yield consists of potentially nutritive dry matter, and what factors influence this proportion, its composition, its loss during cooking, and the quality of the cooked tubers? Finally, how may the crop best be stored if it is to retain a maximum of its nutritive value and palatability?' This is an ambitious programme and it is scarcely surprising that some parts of the book have been accomplished more successfully than others.

Mr Burton has read widely and writes easily. Each of the ten chapters is accompanied by an average of sixty references, so that the treatment accorded any particular piece of work is necessarily short. Nevertheless, in most instances, the essence of research papers has been successfully abstracted and combined in a story which is much less disjointed in its effect than many reviews of this character. The result is that the book is a mine of information and at the same time a useful source of reference for tracing past work on almost every aspect of the potato.

The most detailed treatment is given to the chapters dealing with the potato in storage and as a source of food. This is Mr Burton's special interest, and the useful contributions that he and his colleagues on the staff of the Low Temperature Research Station at Cambridge have already made to the subject appear mainly in this section of the book. The introductory

chapter on the history of the potato is enlivened with quotations from contemporary documents and makes interesting reading, especially the parts dealing with history in the British Isles.

The book is somewhat less successful when dealing with the potato as a growing crop. For example, in a book of this type, it is surprising to find, in a chapter on varieties, no mention of such departures from the normal type as bolters, wildings and other undesirable variations. Some of the few statistics given are also very out-of-date. Thus it makes a curious impression to look up two of our best-known varieties, Arran Banner and Arran Pilot, and to read only that the former comprised 5% of the maincrop acreage in 1935 and the latter 11% of the first early acreage in 1938. The 1946 figures, which one might reasonably have expected in a book published in 1948, were 8% and 45% respectively. However, these are minor criticisms and the fact that this section of the book also is fairly well documented will probably mean that it will be referred to more frequently than the book by Whitehead, McIntosh and Findlay, which, though giving a rather fuller treatment of varieties and cultivation methods, gives no literature references on these subjects.

The weakest section of the book is the chapter on diseases. The author seems here to have been uncertain of his aim, which as expressed in the preface was to estimate the influence of disease on yield. This seems to have become overshadowed, at least in parts of the text, by the inclusion of rather sketchy summaries of the life histories of some of the causal organisms. The author's unfamiliarity with this part of his subject is revealed in many little ways, such as in the explanation suggested for the spread of wart disease (which was due much more to the variety Arran Chief than to Up-to-Date). And although in the rest of the book the emphasis is on potatoes in Great Britain, in the chapter on diseases there are many extracts from American literature (e.g. on stem canker) which have little relevance to conditions in this country. In the paragraph on stalk break there is nothing to indicate that this disease is a very rare one in England, whereas no mention is made of diseases such as black leg, powdery scab, and others which are considerably more important. Scientific workers interested in diseases of the potato will find in Whitehead, McIntosh and Findlay's book a much more balanced account of the pathology of the crop. One thing pathologists here will particularly regret to see, namely, that Holmes's binomial nomenclature for viruses has now been introduced into the literature on this side of the Atlantic.

Notwithstanding its defects on the plant pathology side, this book will be found a useful summary and guide to present knowledge of the potato—particularly of the potato from the time of harvesting until the time of eating. G. GRAHAM SAMUEL

The Spread of Virus Diseases in the Potato Crop. By J. P. DONCASTER and P. H. GREGORY. Pp. viii + 189. Agricultural Research Council Report Series No. 7. London: His Majesty's Stationery Office. 1948. 5*s*.

Previous investigations of virus spread in the United Kingdom have been carried out in areas regarded as suitable for seed-potato production; the survey by Doncaster and Gregory is the first to cover districts where degeneration is rapid. In the study of ecological problems on the field scale the experiments planned should be simple and flexible and the data collected amenable to tabulation and summary; it is a measure of the careful planning of this work that it has permitted the reduction of a large body of data to such a concise but lucid report. The numerous clearly presented tables and diagrams add greatly to its value.

Chapter 1 deals with the development of the potato plant, the time of formation and rate of growth of tubers, and the effects on yield of spacings, sizes of seed, dates of planting and harvesting. Apart from their value to research workers, the results of this work should be of great importance to those concerned with the practical side of seed-potato growing.

The next three chapters describe the study of the potato aphids mainly in the east and south-eastern parts of England. Chapter 2 describes observations on the overwintering of the four

main species encountered, *Myzus persicae* Sulz., *Aphis rhamni* Fonsc., *Aulacorthum solani* (Kltb.) and *Macrosiphum solanifolii* (Ashm.), particular attention being paid to *Myzus persicae* and *Aphis rhamni*, as they are known to be important vectors of rugose mosaic and leaf roll.

The authors conclude that overwintering of *Myzus persicae* on fruiting peaches is more important in the east and south-east of England than in the milder districts of the west and south-west. Their observations do not appear to support the opinion of other workers regarding the overriding importance of savoy cabbages amongst the Brassicae. It has, however, been frequently observed by the reviewer that although 'smooth'-leaved Brassicae become heavily infested with *M. persicae* in the autumn, the aphids on them do not appear to be able to withstand severe weather. The link between the summer and winter populations of *M. persicae* is still incompletely understood and merits further study.

The history of the summer aphid population, which is described in Chapter 3, agrees well with that described by earlier workers. The main differences are connected with the relative importance of the winter hosts, the generally heavier initial infestation of the crops, and the higher peak counts which have been recorded; several of the peak counts are comparable to those found in seed-producing districts. The seasonal and annual fluctuations in aphid numbers are discussed in detail. Valuable data are given correlating aphid counts with the possible sources of immigrants, and in later chapters, full use of this is made in the studies on the spread of rugose mosaic and leaf roll. *Aphis rhamni* was the predominant species on potatoes in 1940, 1941 and 1942 in east and south-east England, but virtually absent in 1943, 1944 and 1945; however, this species does not appear to be an important vector in the field, although laboratory studies have shown it to be at least as efficient a vector as *Myzus persicae*. The explanation suggested results from a study of the behaviour of the two species under field conditions; briefly, an alate *M. persicae* tends to stay on a leaf only long enough to produce a single larva, whereas *Aphis rhamni* tends to produce practically all its offspring on the same leaf. It is also suggested that *A. rhamni* may occur in cycles, but it will take many years to work out the cycle, and it is to be hoped that these observations will be continued. Another observation of fundamental importance is the tendency of *Myzus persicae* to settle on the outer parts of the field, suggesting very strongly that there is a positive attraction to the potato plant, and it was noted that larger plants appear to attract more aphids than small plants. The importance of the former tendency is that when virus is introduced into a crop from a neighbouring field the outer rows will suffer most. Chapter 4 describes the trapping of alate aphids; the trap catches have often agreed closely with aphid counts. On the whole the authors agree with earlier workers regarding the importance of the various weather conditions which influence the flight of *M. persicae*.

Chapters 5, 6 and 7 deal with the increase and the spread of rugose mosaic and leaf roll, and with experiments on roguing. In their planning and execution these experiments set a very high standard. By means of well-designed plots, containing healthy plants (Majestic in most cases) and diseased plants in known positions, supplemented by careful field sampling, the authors demonstrate the pattern of virus spread. As a result of the co-operation of numerous workers in England and Wales, it was possible to replicate these plots at several centres. The results are clearly shown by a series of excellent diagrams and tables, and they indicate that in good seed-producing areas the amount of spread was less than in other areas, and what, perhaps, is of greater interest, that the most important sources of infection are those within the crop itself. These trials also included date-of-lifting experiments, and it is concluded that, as a result of the earliness of virus spread, early lifting is not a sound method of obtaining healthy seed. Experiments on the distance of spread confirmed the importance of internal infectors, and roguing experiments the importance of the early spread. Roguing seemed to suggest that one of the main differences between good and bad seed areas is that spread is early and rapid in the one and later and slow in the other. There are also fundamental farming differences which may operate, e.g. the seed-growing areas in north Wales are primarily stock-rearing districts; before the war the arable acreage was small, and even

at the height of the ploughing campaign potato fields were small and neighbours' fields not often adjacent, with the result that, provided healthy stocks are planted, the danger from outside infection is not so great as it would be in an arable district.

Chapters 8 and 9 deal with fumigation experiments and the survival of volunteers. Nicotine fumigation kills aphids, but has no marked effect on virus spread. There is an astonishing survival of volunteers in crops other than potatoes (except between drills it is difficult to count them in potatoes), but it was found that these volunteers were generally healthy; it is suggested that they may be much more important in relation to other diseases such as potato root eelworm. It would be interesting to know whether a similar survival of volunteer potatoes is found in western and northern districts.

In the concluding chapter the authors find it impossible to reach a firm conclusion on the correlation between virus spread and vector numbers. There was a slight positive correlation between counts of *M. persicae* and the spread of leaf roll, but no apparent correlation between *M. persicae* and rugose mosaic. Whitehead has shown, however, that in north Wales there is a decided correlation between the numbers of *M. persicae* and the degeneration of potatoes. The methods used have, however, been rather different. Doncaster and Gregory have tried to correlate the carefully measured amount of spread in individual crops with the observed aphid populations, whereas Whitehead's results were based upon the overall increase in virus diseases in all seed-potato stocks in a number of districts in most of which aphid counts were made at a relatively small number of stations. It is, however, abundantly evident that the correlation between virus spread and the vectors is a complicated problem. It would be useful if such a correlation could be used in the certification of seed potatoes, and it is hoped that this subject will continue to be studied.

The special role of *M. persicae* and the bearing of this work on seed-potato production are discussed, and valuable suggestions are added for future work.

Pages 144–81 consist of appendices containing summaries of the numerical data accumulated during the survey, and these tables are followed by a carefully compiled index.

Altogether, this work, which has been so admirably presented, is an invaluable contribution to the literature on this subject and will be eagerly read by all workers on potato aphids and virus diseases.

F. H. JACOB

Plant Diseases. By F. C. BAWDEN. Pp. x + 206, Plates 30. Edinburgh and London: Thomas Nelson and Sons, Ltd. 1948. 7*s*. 6*d*.

The volumes of Nelson's Agriculture Series provide unusual value, quantitatively in supplying 75,000 words and thirty plates for 7*s*. 6*d*., qualitatively in the high standard of the texts. The choice of Mr Bawden to cover the field of plant diseases ensures an individual and highly perceptive survey in which the wood is never hidden by the trees.

An introductory chapter on causes of disease in plants, enlivened with a convincing refutation of the 'compostolic' creed, is followed by chapters on the general characters of seed-borne and of soil-borne diseases; chapter 4 covers those that are air-borne and insect-borne. The section on insect-borne diseases, incorporating a lucid exposition of the distinction between the 'persistent' and the 'non-persistent' viruses, might well have stood separately. Chapter 5 discusses alternate and alternative hosts of both fungus and virus infections, and the succeeding pages sum up our present knowledge of diseases caused by malnutrition and other unfavourable circumstances. Chapter 7 unravels some of the complexities of the effects of nutrition and environment on disease expression; the next chapter surveys the interrelationships of genetics and plant diseases. Finally, the principles of plant-disease control are reviewed.

Among the outstanding merits of this book are the surefootedness with which the author establishes the balance between its diverse components and the lucidity of his style. The search for a flaw in Mr Bawden's limpid prose is unrewarding, although possibly there is a slight haziness in the locution 'parasites with long-lived reproductive organs'. More frequently one's

eye is caught by the glitter of an aphorism such as this: 'It is often said that agriculture and horticulture afford a natural and healthy life. For the people engaged on them, this may be true, but for the crops, nothing could be further from the truth.'

Writers who peer closely into the technological details of diagnosis and treatment of plant diseases are valuable but not rare: Mr Bawden stands out as the first English author sufficiently far-sighted to direct our gaze to a wider prospect—the whole panorama of the science of phytopathology.

R. W. MARSH

Organic Form and Related Biological Problems. By SAMUEL J. HOLMES. Pp. vii + 169. United States of America: University of California Press; Great Britain and Ireland: Cambridge University Press. 27*s.* 6*d.*

This book is an inquiry into the problem of organic form—the elusive central problem of biology. It is an attempt to find an explanation, compatible with the known sciences, for the ability of organisms to maintain and regenerate the relative proportions of their parts and to preserve the normal balance of their physiological functions. The approach to all the problems discussed—the ordered development of the individual, the meaning of recapitulation, the biological nature of cancer, the origin of life—is that of the mechanist who would seek an explanation of the baffling phenomena of life in the integration at a higher level of properties which in themselves we feel we understand. The book is written in essay style; it is not a work of reference, but calls for careful reading and reflexion. A book of that kind must be the product of deep thought and must be well written if it is to be worth reading. S. J. Holmes has been thinking and writing upon these problems for more than forty years. His book is honest and unprejudiced, full of wise saws and modern instances, and he writes English which it is a pleasure to read.

V. B. WIGGLESWORTH

Les Richesses de la Mer. By NOEL BOUDAREL. Pp. 548, fig. 1020 (Encylopedie Biologique, XXIX). Paris: Paul Lechevalier. 1948. Prix 1500 fr.

The book, which starts with the origin of the earth and ends with the preservation of sardines, tries to touch on every aspect of pure and applied marine biology. With such a tremendous field to cover it is not surprising that the book fails in its purpose. The general reader, for whom it is obviously meant, will obtain a very unbalanced view of marine biology. This is a great pity, because the efforts of the author to provide so comprehensively illustrated a book deserved more success.

The chapters dealing with the marine phyla vary considerably in quality. For instance, only two polychaetes are described, yet the descriptions are inaccurate. In the case of *Arenicola marina* (referred to as *A. piscatorum*) the burrow is stated to be an open 'U', and the figure shows twelve instead of thirteen segments with branchiae. In view of the fact that in the *Faune de France* the number of branchiae is used as a character for separating species this slip is inexcusable. The case of *Nephthys Hombergii* (referred to as *Nephtys Hombergi*) is still more surprising, for the drawing does not even represent *Nephthys* sp. The chapter on molluscs is another example of the author's carelessness. He categorically states that 'nous donnerons une liste figurée (fig. 340–613) de tous les mollusques qui vivent sur nos cotes. . . .' Yet the Nudibranchs are not even mentioned. Whilst the illustrations of organisms are technically good, minor inaccuracies render several of them very misleading.

The author can be forgiven for not being up to date with all changes in nomenclature, but surely a member of the staff of the Museum de Histoire Naturelle should know that the authority for a specific name does not always require brackets.

Fortunately, practically half the book is devoted to fish and methods of fishing. In spite of some confusion over the term 'cartilaginous fishes', these sections do much to recover the reputation of the book. Those chapters dealing with the many types of nets and traps used around the coasts of France are of particular interest and are admirably illustrated.

J. B. CRAGG